Laboratory Medicine Practice Guidelines

Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice

Edited by Roland Valdes, Jr., Deborah Payne, and Mark W. Linder

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Presents

LABORATORY MEDICINE PRACTICE GUIDELINES

LABORATORY ANALYSIS AND APPLICATION OF PHARMACOGENETICS TO CLINICAL PRACTICE

EDITED BY
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Chapter 1

General Introduction and Scope

Roland Valdes Jr

OBJECTIVES OF THE LMPG FOR PHARMACOGENETICS

The objective of this document is to establish practice guidelines for application of pharmacogenetics (PGx) in the practice of laboratory medicine.

This document will present evidence supporting the application of PGx testing to general clinical practice and specific areas of medical practice. We will provide guidance for PGx testing in specific clinical settings; define potential links in the roles of PGx and therapeutic drug monitoring in clinical settings; formulate recommended guidelines for clinical laboratories introducing pharmacogenetic tests; and provide in vitro diagnostic companies guidance on clinical assays and their performance characteristics in PGx testing.

APPROACH TO GRADING THE EVIDENCE

Regarding the evidence base for this particular set of guidelines involving PGx, it is noteworthy that published studies have shown poor correlations between the methodological quality used in preparing guidelines in general and the validity of recommendations provided (1). Thus, in disciplines which are rapidly evolving (eg, PGx) and where the evidence is as yet uncertain, the need exists for documents offering the opportunity to include a series of balanced thoughts behind the recommendations and to also identify gaps in the knowledge and include different options when the evidence is unclear or lacking. Thus, robust recommendations can be forwarded even without a more desirable rigorous evidence-based approach typical of more well-established applications. In fact, as indicated by Burgers (2), guidelines are particularly needed in disciplines with areas of uncertainty compared with more established applications as may be readily available in textbooks.

NACB recommends the use of grades to determine the strength of evidence for practice guidelines, as follows:

A. The NACB strongly recommends adoption; there is good evidence that it improves important health outcomes and concludes that benefits substantially outweigh harms.

B. The NACB recommends adoption; there is at least fair evidence that it improves important health outcomes and concludes that benefits outweigh harms.

C. The NACB recommends against adoption; there is evidence that it is ineffective or that harm outweighs benefit.

I. The NACB concludes that the evidence is insufficient to make recommendations; evidence that it is effective is lacking, of poor quality, or conflicting; and the balance of benefits and harms cannot be determined.

NACB grades the quality of the overall evidence on a 3-point scale, as follows:

I. Evidence includes consistent results from well-designed, well-conducted studies in representative populations.

II. Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence.

III. Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information. Expert consensus is the basis for the recommendation.

GENERAL SCOPE OF PGX AS A DISCIPLINE IN LABORATORY MEDICINE

Pharmacogenomics and, more specifically, PGx deal with the use of information derived from analysis of gene variations for purposes of guiding the use of medications and related therapeutics. Typically, this discipline has two functional component arms that link pharmacology to genetics. These arms are best divided into predicting how drugs are processed by the body (pharmacokinetics) and how they interact with receptors to cause an anticipated response (pharmacodynamics). Typically, pharmacokinetics is strongly linked to biotransformation of drugs by metabolic processes (eg, by the liver) and their subsequent elimination by kidney function. In contrast, pharmacodynamics deals with understanding the interaction of drugs with receptors and the subsequent response, albeit some biotransformation may be involved. Thus, the former concept as related to PGx testing in laboratory medicine is usually associated with predicting the biotransformation of a drug by detecting specific genetic variants that control aspects of pharmacological response.

Several compelling developments help drive the need for guidelines such as those presented in his document:

- Rapid flux of knowledge (guidance needed)
- Unclear evidence for clinical application (ie, evidence of benefit)
- Need for more education
- Transition to more complex technology (research to IVD and clinical applications)
- Confusion among payers and regulators
- Changing role of clinical laboratories (central to transition)

The clinical laboratory plays a critical role in moving the clinical application of PGx forward. The optimum role of the clinical laboratory relative to PGx might be summarized as follows:

- Establishing genetic profiling strategies to maximize sensitivity and specificity of predicting phenotype
- Developing methods to reduce testing cost and technical difficulty
- Providing availability of testing
- Educating the end-users of PGx test results
- Overcoming perceived barriers to genotyping

The following guidelines therefore serve as a guide toward the future development of PGx as a discipline in laboratory medicine.

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Chapter **2**

Pharmacology and Population Genetics Considerations and Their Applications in Pharmacogenetics

Gualberto Ruaño and Roland Valdes Jr

GENERAL INTRODUCTION

This Chapter provides a primer in the principles of drug metabolism and population genetics with sufficient basis for understanding how the concepts of genetics are applied in the development and application of pharmacogenetic testing as a discipline.

Questions for consideration are:

a) What are the essential elements of drug pharmacokinetics and pharmacodynamics necessary to understand the application of pharmacogenetics in laboratory medicine?

b) What is the cytochrome P450 system and what are examples of the relevant allele frequencies of these components (eg, *CYP2D6*, *CYP2C19*, and *CYP2C9*)?

c) What are key considerations and recommendations for statistical sampling of the indicated alleles in populations?

RECOMMENDATIONS

a) What are the essential elements of drug pharmacokinetics and pharmacodynamics necessary to understand the application of pharmacogenetics in laboratory medicine?

Introduction to Drug Metabolism, Pharmacokinetics, and Pharmacodynamics

The Half-Lives of Drugs in Blood

The use of therapeutic drug monitoring (TDM)—or "management," as it is now often referred—has revealed wide differences in the plasma half-life of commonly used medications (Table 1 adapted from Weber (1). In addition, multimodal distributions of drug concentrations in blood of patients ingesting comparable amounts of drug have been reported (1). Genetic variants associated with drug-metabolizing enzymes have been implicated as the basis for these observations, particularly as they pertain to variation in the processing of drugs (pharmacokinetics variation) by individuals.

Table 1. Person to Person Variation in Plasma Elimination Half-Life

Drug	Half-Life (hours)
Isoniazid	0.5-7.5
Tolbutamide	4-10
Phenytoin	10-42
Warfarin	15-70

Receptors and the Concentration of Drugs in Blood

The theory of "receptor occupancy" is central to understanding drug action and is linked to the effective concentrations of a drug in blood. The relationship between the concentration of a drug in blood and the response caused by that drug is shown in Figure 1 (A and B) for two conditions. Panel A depicts the number of available receptors and panel 1B shows the affinity of the receptors. Both situations might be affected by genetically based contributions; for example, a genetic variant in the promoter region may reduce the number of receptors or a genetic variant in the coding region may affect the affinity of the receptors. A second relationship to understand is the one between the dose of a drug ingested and the concentration (drug burden) of the drug in blood. The descriptive elements that dictate the concentration-time curve or area under the curve (AUC) of a drug in blood are shown in Figure 2 (left and right panels), including the subsequent action on the target cells (adapted from Weber (1)) and hence the link to the importance of receptor mediated responses.

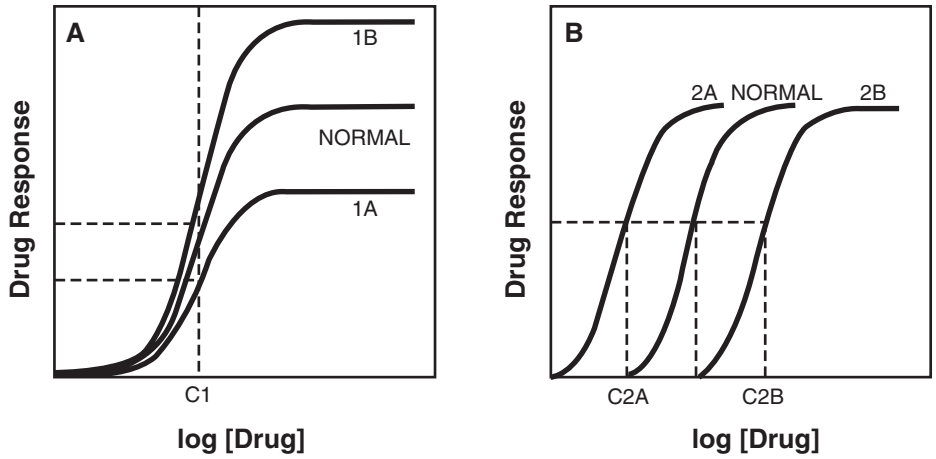

Figure 1. Panel A shows the relationship between the number of receptors and drug response, where 1A is a low number and 1B is a high number of receptors. Panel B shows the relationship of drug response to affinity of receptors, where 2A is a high affinity receptors and 2B is a low affinity. Note that the 50% effective drug concentration C2A is lower for a high affinity receptor compared with concentration C2B (adapted from Weber (1)).

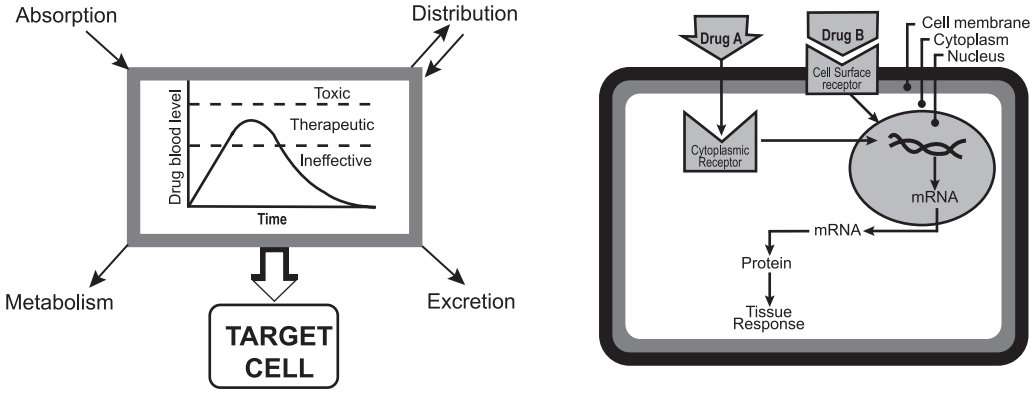

Figure 2. The left panel shows the area under the curve (AUC) for a drug dose and the elements controlling the shape and magnitude of the curve; typically, absorption, distribution, excretion, and metabolism. The right panel shows the subsequent interaction of the drug with a cell surface receptor or a cytoplasmic receptor. The affinity and number of the receptors are linked to the discussion of Figure 1 (adapted from Weber (1)).

Liver as a Drug-Metabolizing Organ

Although other organ systems (eg, intestines) are known to metabolize medications, the liver is by far the principal organ responsible for processing and providing major pathways for the biotransformation of many ingested medications. The concentration- time curve shown above is controlled by a combination of drug bioavailability, biotransformation (metabolism), and subsequent elimination by the kidney.

Phase I and Phase II Drug Metabolism

The metabolic process is generally described as having two phases based on the nature of the transformation. Phase I involves an oxidative process, whereas phase II metabolism is generally conjugative. Table 2 lists a few of the relevant enzymes as examples in each category.

Genotyping and Drug Dosing Requirements

At a practical level, one might ask if there is evidence that the concentration-time curve in response to drug dosing is affected by genotype linked to a pharmacogenetic variant (ie, test result). Figure 4, from a review by Brockmoller (3), shows a relationship between the AUC and the genotype for *CYP2C19* relative to the drug omeprazole. It serves as an example of the marked effects that a genetic variant may have on the important dynamics of drug metabolism and accumulation in blood. Note that a homozygote *CYP2C19*2/*2* has an AUC almost 9 times higher than a *CYP2C19*1/*1* homozygote (wild-type; ie, normal genotype). In addition, a heterozygote also has a reduced elimination of this drug manifested by a two-fold higher AUC compared with normals.

Adverse Drug Reactions

Systematic studies involving review of published literature indicate that adverse drug reactions (ADRs) are indeed prevalent and associated with costly hospitalizations (4). Such studies indicate that on average more than 2.2 million such events each year are frequently observed in the United States, and that a substantial fraction (>100,000 per year) involve subsequent mortality. In addition, published

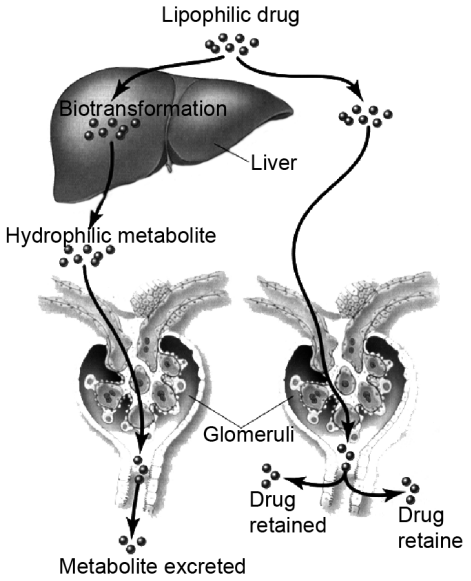

Figure 3. Transition of lipophilic drugs. Lipophilic drugs are biotransformed in the liver for subsequent excretion by the kidney. Without biotransformation a lipophilic compound is typically reabsorbed (2).

Table 2. Metabolic Process
Phase I (oxidative)
Cytochrome P450s
Monoamine oxidase
Alcohol dehydrogenase
Aldehyde dehydrogenase
Dopamine B-hydroxylase
Phase II (conjugative)
N-acetyl transferase (*NAT*)
Thiopurine methyltransferase (*TPMT*)
UDP glucuronosyltransferase (*UGT*)

literature reviews show a link between ADRs, classes of drugs, and genetic variants of key metabolizing enzymes. One review suggests that 59% of the drugs associated with reported ADRs are metabolized by liver enzymes for which genetic variants have been described, compared with only 20% for the randomly selected drugs (5).

From the perspective of this guideline, it may be important to suggest that the definition of ADR be expanded to include other situations not typically considered but that can lead to costly medical intervention, including complications due to or leading to the loss of drug efficacy, reduced compliance, the misclassifications of physician ordering patterns, psychological implications to patients, and other complications leading to reduced health care efficiency as well as increased costs. Many of these situations are known to occur, but are not now classified as important as an ADR, which may require immediate hospitalization, or cause morbidity or mortality. Nevertheless, these less well-defined conditions do lead to costly and unnecessary medical interventions. This may be of practical importance due to the advancing age of worldwide populations and the increased number of individuals taking multiple drugs (polypharmacy; Fig 5).

In any event, substantial documentation exists to address the efficacy of major drugs used to treat several important diseases and their dependence on a pharmacogenetic component (7). An example of this concept is the use of disease-preventive therapies (eg, tamoxifen), which may not have biological markers for efficacy but require appropriate concentrations of drug in blood.

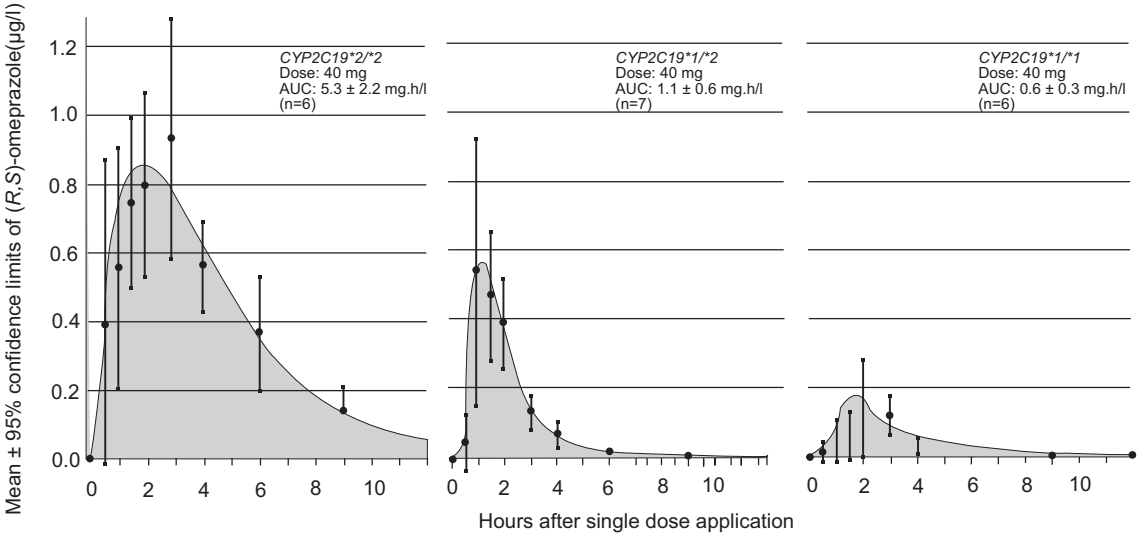

Figure 4. Relationship between the AUC and the genotype for CYP2C19 relative to the drug omeprazole. (3)

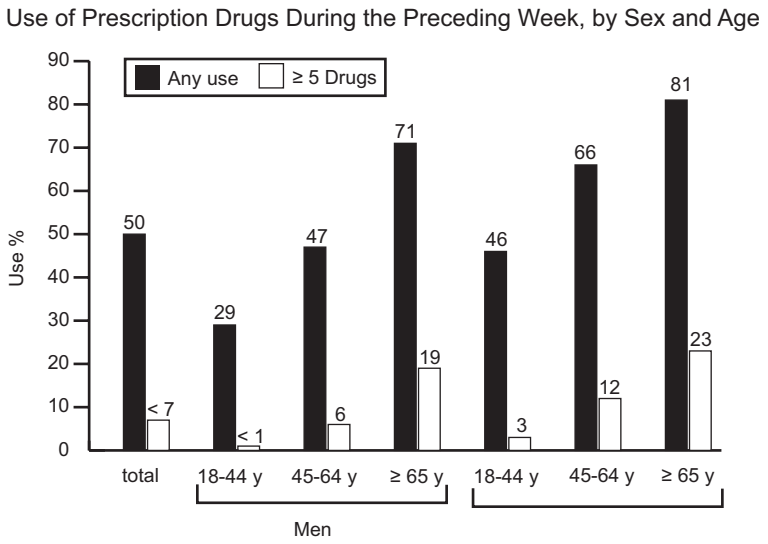

Figure 5. Graph shows the percentage of individuals by sex and age who are taking prescribed medications. Note that for individuals older than 65 years—upward of 70% are using more than five medications (ie, polypharmacy; Adapted from Kaufman et al (6)).

b) What is the cytochrome P450 system and what are examples of the relevant allele frequencies of these components (eg, *CYP2D6*, *CYP2C19*, and *CYP2C9*)?

Introduction to the Cytochrome P450 system

Cytochrome p450 is a family of heme-containing enzymes that catalyze the conversion of lipophilic substances into hydrophilic molecules which can then be excreted by kidneys into urine. It represents a major part of the body's powerful detoxification systems. The cytochrome p450 system metabolizes endogenous and exogenous substrates through a variety of reactions including epoxidation, N-dealkylation, O-dealkylation, S-oxidation, and hydroxylation. Exogenous substances (products ingested or absorbed) include not only pharmaceutical compounds given as therapeutic drugs, but also foodstuffs and dietary components, and occupational pollutants and industrial chemicals. The cytochrome p450 mixed-function mono-oxygenase system is probably the most important element of phase I metabolism in mammals. More than half of all drugs are primarily cleared by the cytochrome p450 system (8-10). As a group, these enzymes are often referred to as drug metabolizing enzymes (DME). The activity of these enzymes is modulated by a variety of factors including age, diet, concomitant medications as well as genetic variability.

The cytochrome p450 system has evolved into a gene family and expanded into multiple chromosome loci, each with tandem arrays of genes, and each gene with substantial polymorphism. This system is an illustration of gene expansion, multi-gene families, and allelic functional variation. Genomics has supplied a rich resource of gene mapping data as well as the individual variants in each gene at the single nucleotide polymorphism (SNP) and chromosome locus levels. The 57 cytochrome p450 isoforms now known in humans, along with the hundreds of genetic variations, have produced a large set of biomarkers predictive of susceptibility to specific toxins. The fact that the pharmaceutical industry routinely includes an assessment of the main metabolic pathways of a candidate drug to derive clinical pharmacological correlations is indicative of the importance of this knowledge (11,12). In addition, certain cytochrome p450 alleles can also be disease susceptibility markers. For example, some are known to be implicated in detoxification or activation of environmental toxins and variants associated with cancer risk (eg, Agundez et al (13).

The nomenclature used to categorize the variant alleles of the cytochrome p450 enzyme system has been defined by various organizations (14). In general, the descriptors rely on the hierarchy of the genetic structures involved in the construction of the enzymes. Figure 6 describes one common basis for a nomenclature and the one used throughout these guidelines.

Of the cytochrome P450 enzymes described to date and most closely related to clinical applications through pharmacogenetic testing are the CYP2D6, CYP2C9, and CYP2C19 enzymes. Factors such as high prevalence of variants, and in the case of CYP2D6 full-length gene duplications, in human populations and wide range of therapeutics metabolized by these enzymes render them among the most relevant drug metabolizing enzyme for PGx diagnostics. Other enzymes or receptors mentioned throughout these guidelines, such UGT1A1 or VKORC1, are described as they are presented in text.

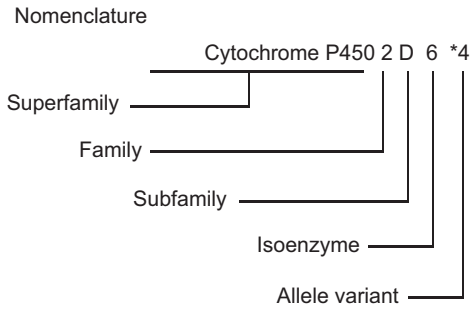

Figure 6. Nomenclature and the cytochrome P450 system.

Recommendation 1
We recommend that the present nomenclature used to describe the phenotypes (eg, extensive metabolizer or ultrarapid metabolizer) associated with a particular genotype (eg, wild-type, homozygote, mutant) be reconsidered so as to provide a more medically relevant pharmacogenetic nomenclature (B-II).text text

Rationale

The terminology now being used to describe phenotypes such as extensive, rapid, intermediate, or ultra-extensive metabolizers and the terminology presently used to describe genotypes, such as wild-type, should be re-evaluated in place of a more clinically descriptive nomenclature better fitted to more standard medical practice. For example, "wild-type" should not be used to describe the more common alleles since in some cases the discovery of new variant" alleles are found to be more common than previously thought (15). Nomenclature, such as "mutation", does not seem practical to be used in reference to an altered genotype and the word "variant" is a better and recommended alternative. Appropriate professional organizations should reconsider establishing a more standard nomenclature now that pharmacogenetics has more rigorous and better-defined medical applications.

However, in view of more common and presently utilized nomenclature and to reduce confusion throughout these guidelines and the references, we will continue to use the more prevalent nomenclature as necessary.

CYP2D6

The variability of metabolism of debrisoquine and sparteine was discovered in the late 1970s (16) in what is now the classic example of phenotypic variability ascribed to isoenzymes, which we now know result from gene polymorphism. The metabolic variability could be traced to the *CYP2D6* gene. This gene was found to be hypervariable, which required analysis of multiple SNPs as well as of its deletion and full-length gene duplication in some individuals (17,18). In what may be an example of adaptive evolution, hypervariability on this gene is potentially advantageous, which would be consistent with the need to process and detoxify different substances in various environments. At least 70 alleles have been described for this gene (19) and the phenotypic characteristics of their diploid constitutions in humans have been ascertained (20). This variability has been historically divided into four phenotype categories (Table 3): ultra-rapid, extensive, intermediate, and poor metabolizers. However, there is no agreement on how to define an intermediate metabolizer. One definition provides that a person with one active allele and a null allele is an intermediate metabolizer (18). The other definition for an intermediate metabolizer is a subject with a null allele and one with low activity (eg., *CYP2D6*10*; 21). Although this issue remains unresolved, recent progress has been made toward improving the fine tuning of genotype-phenotype correlations in order to provide practical clinical significance (18,22,23). The clinical perspective on this is more closely addressed in the section below on Clinical Considerations.

Previous studies have demonstrated marked ethnogeographic variance in the distribution of cytochrome p450 enzymes throughout the world. For the *CYP2D6* gene, in particular, the allele frequencies show significant diversity, and representation of alleles are known to have multi-continent segregation. The non-European common alleles most likely observed in an urban population with the United States are alleles *CYP2D6*17* and *CYP2D6*10* and the gene duplication. Allele *CYP2D6*17* is reported to be found predominantly in individuals of African ancestry. Allele *CYP2D6*10* is found predominantly in individuals of Asian origin. The gene duplication allele is found predominantly in individuals of origin traceable to Northern Africa and the Middle East (24,25). Studies in major urban centers may

Table 3. Metabolizer Status, Influence on Drug Dosing, and Population of Cytochrome P450 Phenotypes in Surveys of Individuals of Western European Ancestry

	Prevalence (%)		
Metabolism Status and Influence on Drug Dosing	***CYP2C9***	***CYP2C19***	***CYP2D6***
Poor metabolizers are at increased risk of drug-induced side effects due to diminished drug elimination or lack of therapeutic effect resulting from failure to generate the active form of the drug. Individuals have a deficiency in drug metabolism.	3	4	10
Intermediate metabolizers may require lower than average drug dosages for optimal therapeutic response. In addition, multiple drug therapy should be monitored closely.	30	38	40
Extensive metabolizers represent the norm for metabolic capacity and therefore posses the full complement of drug metabolizing capacity. Generally, extensive metabolizers can be administered drugs which are substrates of the enzyme following standard dosing practices.	67	58	49
Ultra-rapid metabolizers have increased metabolic capacity and may require an increased dosage due to higher than normal rates of drug metabolism. Simultaneously treating with medication that inhibits metabolism has also proven effective.	0	4 to 18	1

NOTE. We address nomenclature in recommendation 1.

show the inherent diversity of populations in the service area, suggesting the recognition of DNA typing as a major tool in dealing with the diversity of our patients. In any event, it should be noted that 20% to 25% of all drugs are subject to metabolism by CYP2D6, and that, while more than 70 *CYP2D6* variants have been identified, several laboratories have reported that most poor metabolizers can be detected by screening for up to five alleles (26).

The distribution and frequency of these phenotypes is dominated by reliance on biogeographic ancestry (ie, race) as a factor, which may be restrictive for the modern practice of pharmacogenetics. Published estimates routinely point to a 10% frequency of the poor metabolizer phenotype in individuals of Western European ancestry ("Caucasians"; where the *CYP2D6*4* allele is common; 25), and a frequency of only 1% in individuals of Far East Asian ancestry (27). Intermediate metabolizers account for 40% of the Caucasian population. The extensive metabolizer phenotype is the most common in Caucasian populations, accounting for one half of the population. Differences in response among populations of different ethnogeographic origin can often provide clues to the influence of genetic factors on individual susceptibility to the efficacy and toxicity of specific drugs. Perhaps the best-known example is primaquine sensitivity due to G6PD deficiency among African and Mediterranean peoples (28). Another important example is the remarkable difference in sensitivity of Caucasians and Japanese to the adverse effects of ethanol attributed to ethnic differences in the variant isoforms of the enzymes that metabolise ethanol and acetaldehyde (29). High allele frequencies of *CYP2D6*10* and *CYP2D6*41* have been noted among Middle Eastern ethnic populations (30). Additional examples of ethnogeographic variation associated with *CYP2D6* and *CYP2C19* are discussed below. The importance of *CYP2D6* metabolism particularly as related to ultra-rapid or poor metabolism is also noted for the pro-drugs such as codeine and tramadol which are metabolized to their active compounds by CYP2D6 activity.

CYP2C9

A host of drugs, including Fluoxetine, sertraline, warfarin, acenocoumaral, tolbutamide, losartan, phenytoin, and glipizide, are metabolized by this enzyme (31). Evidence suggests that fluoxitine and sertraline are only partly metabolized by CYP2C9 and that other cytochrome P450 enzymes including CYP2D6 play a role (32,33). The most common polymorphisms in *CYP2C9* are *CYP2C9*2* (Arg-144-Cys, C-430-T SNP) and *CYP2C9*3* (Ile-359-Leu, A-1075-C SNP). Both are functionally deficient allele studies (34). Both *CYP2C9*2* and *CYP2C9*3* show reduced catalytic activity (increased Km) and /or decreased maximum rate of metabolism (decreased Vmax) in vitro. The *CYP2C9* gene is of great relevance to cardiovascular medicine as it metabolizes warfarin, a standard treatment for thromboembolism. For in vivo correlations, *CYP2C9*3* heterozygotes and homozygotes were demonstrated to have reduced (S)-warfarin clearance by 66% and 90%, respectively. Also, *CYP2C9*2* or *CYP2C9*3* carriers require lower maintenance dose of warfarin, and experience a first bleeding event sooner with standard unadjusted dosages, consistent with a poor or intermediate metabolizer phenotype. Finally, *CYP2C9*3* homozygotes are poor metabolizers for warfarin, acenocoumaral, tolbutamide, losartan, phenytoin, and glipizide.

CYP2C19

The *CYP2C19* polymorphism was first identified as S-mephenytoin hydroxylation polymorphism (35). Diverse pharmacologically important therapeutic agents, such as antidepressants (ie, citalopram, tricyclic), anti-epileptics, and proton pump inhibitors have been identified as substrates. Several variant alleles of *CYP2C19* cause the expression of inactive enzyme. The variants are alleles *CYP2C19*2* to *CYP2C19*8*, but other more rare variants from *CYP2C19*4* to *CYP2C19*8* have been described. The *CYP2C19*2* and *CYP2C19*3* alleles account for greater than 95% of deficient alleles in most populations studied to date (36). Heterozygosity for any of these alleles requires drug dosage adjustment (17,18). Similar to *CYP2D6*, the variability in *CYP2C19* allele frequencies varies significantly between ethnicities. The frequency of individuals who have one or more deficient *CYP2C19* alleles is 2% to 5% in Caucasian and of 13% to 23% in Asiatic populations. As indicated in Table 3, studies indicate that *CYP2C19* is also associated with rapid metabolizer phenotypes (37) the frequency of which was population dependent (18% in Swedish and Ethiopian populations and 4% in Chinese).

c) What are key considerations and recommendations for statistically sampling of the indicated alleles in populations?

Recommendation 2
To identify a regional difference between published allele frequencies and a specific service population, it is recommended that a survey of 100 to 150 individuals be tested (B-II). The need to perform this analysis for each clinical service population is not yet established.

Rationale

The sample size needed to detect a deviation of the mean number of functional alleles is given by

$$n = \frac{\sigma^2 (Z_{1-\alpha/2} + Z_{1-\beta})^2}{2},$$

where σ is the standard deviation (assumed equal between the two samples), Δ is the difference between the means to be detected, α = 0.05 is the significance level, Z is the normal deviate (38), also known as the standard normal quantile function, and 1-β = 0.8 or 0.9 is the desired power. Figure 7 shows the dependence of the detectable Δ on sample size, showing that at the recommended 100 to 150 samples a difference of 0.25 to 0.30 alleles is well within the range of detection. The two most likely reasons for a difference include an ethnogeographic ancestry distinctive to the service population and referral bias of patients with deficient

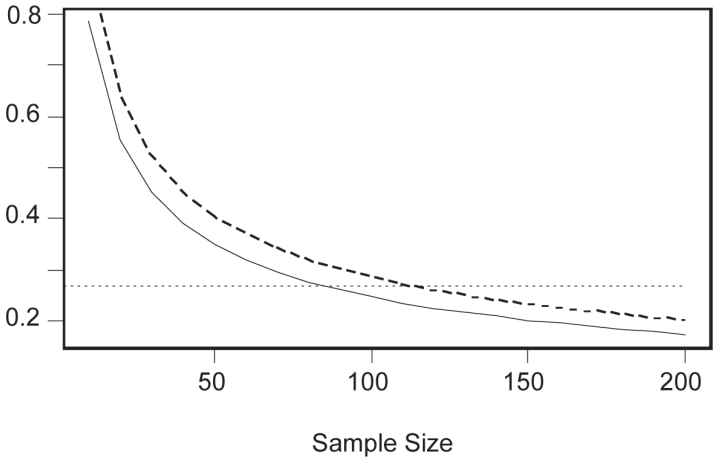

Figure 7. Difference in mean number of functional alleles that can be detected versus the number of patients genotyped. For power of 80% (solid line) and 90% (dashed line). The dotted line is between 0.25 and 0.30 difference. Note: Classical population statistics such as Hardy-Weinberg equilibrium may have limited applicability to a population sample of diverse ethnogeographic ancestry typical of urban populations.

and null alleles to tertiary care centers for medical management complications. For either of these reasons or both, it is desir-able to publish these regional and service-specific surveys. Each center will have specific features pertinent to the population it serves that are of great value for the entire field of pharmacoge-netics as a whole and to the development of the new standard of care based on individualized therapy (39). For a general discus-sion on statistical considerations relevant to pharmacogenetics, please refer to Holford et al (40).

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Chapter 3

Methodology and Quality Assurance Considerations in Pharmacogenetic Testing

Deborah A. Payne and Jeanne Carr

GENERAL INTRODUCTION

When performing pharmacogenetic testing, the laboratory should follow the guidelines established for molecular pathology testing. The specific guideline to follow depends on the laboratory's geographical location. Specifically, laboratories within the United States follow CLIA and the College of American Pathology (CAP) guidelines while some international laboratories should follow guidelines outlined by the International Organisation for Standardisations's Technical Committee (ie, ISO 15189) (1). These guidelines describe numerous quality assurance and quality control issues that can control for problems associated with preanalytical and analytical phases when performing molecular diagnostic tests. It should be noted that, approaches and guidelines for clinical and analytical validation of nucleic acid analysis have been previously described (2-4). The technology used for determining genotype is becoming more complex and will require additional measures to assure reliable and acceptable test results. The goal of these guidelines is to provide recommendations that will facilitate reproducible intra- and inter-laboratory results for pharmacogenetic tests. The supporting data and/or documentation available to the laboratory will determine the extent of additional documentation required by the end user to satisfy each recommendation.

Questions for consideration are:

a) How should discrepancies in results be identified and investigated?

b) What materials may be used to validate pharmacogenetic tests?

c) What methods may be employed to avoid or identify assay interferences?

d) What reference/control material will be used for validation and lot-to-lot quality control?

e) Is proficiency for pharmacogenetic testing different from other molecular diagnostic tests and how often should it be performed?

f) If software is used in interpretation, will it automatically flag extremely rare or unlikely allele combinations?

RECOMMENDATIONS

a) How should discrepancies in results be identified and investigated?

> ***Recommendation 1***
> Analytical sensitivity and specificity should be determined for each assay (A-I).

Rationale 1

Random errors may be identified based on inconsistencies between the clinical phenotype while systematic errors may be identified by trends and comparison of genotype frequencies with the anticipated frequencies. Some platforms may not be able to detect large exonic or gene level insertions and deletions and thus may report a patient as being homozygous normal versus heterozygous. For example, FISH may be able to detect large deletions or full-length gene duplications but not partial gene, complete gene deletions, or small nucleotide changes. Sequencing may not detect a large deletion of one allele but can identify single nucleotide changes.

> ***Recommendation 2***
> When analytical results are inconsistent with clinical information or statistics of patient populations' genotype frequencies, there should be evidence that these potential discrepancies have been investigated (A-I).

Rationale 1

Statistics of patient population frequencies must be maintained to monitor any significant changes or trends in the detection of variants or normal genotypes. These population frequencies

must be obtained from the patient test population and not cited from nonregional published data (5).

b) What materials may be used to validate pharmacogenetic tests?

Recommendation 3
Validation of each pharmacogenetic test should use samples where the genotype has been independently verified. Ideally these materials would be from a renewable and sustainable source. If the error rate has been identified and/or reported for each pharmacogenetic assay, this information should be included in the report/protocol (A-I).

Rationale

Several sources now exist for independently verified reference materials for pharmacogenetic testing (6-9). There is a database available for querying the location of these references materials (10).

c) What methods may be employed to avoid or identify assay interferences?

Recommendation 4
Laboratory validation of various sample types should follow standard guidelines for molecular pathology tests (A-I).

Rationale 1

Control procedures that detect potential inhibitors or interferants for assays that utilize enzymes should be incorporated into the assay. These procedures may involve inclusion of an exogenous target or the assay could be designed so that restriction enzyme or cleavage sites are present for both normal and variant targets. IVD manufacturers may have the responsibility to provide such information to the appropriate regulatory agency for that region (eg, FDA or Conformité Européne a.k.a. European Conformity (CE).

Rationale 2

Extracted nucleic acids are less likely than crude samples, such as specimens from fecal or plasma nucleic acids, to contain inhibitors toward enzymatic amplification or target detection (ie, restriction fragment length analysis or flap enzymatic analysis). Controls that detect potential inhibitors for assays that use enzymes, such as allele specific amplification, restriction enzyme digestion, or cleavase-based assays, should be incorporated into the assay on each run (5).

Rationale 3

The pharmacogenetic test method will determine the quality and size requirements for the extracted nucleic acid to be analyzed. Southern analysis requires larger sizes of nucleic acid with minimal degradation. Methods that utilize enzymatic amplification for the characterization of the genotype may vary for different sources of nucleic acids. Buccal swab samples may contain less nucleic acid than blood samples and may require larger volumes or an additional concentration steps to yield comparable results as blood-derived extracted material. Paraffin samples have size constraints for amplicons compared with whole blood samples, thus preventing the use of long range PCR analysis for haplotyping.

d) What material will be used for validation and lot-to-lot quality control?

Recommendation 5
Assay validation should include whole genomic samples possessing homozygote normal, heterozygote, and homozygote variant, when possible. Assay validation should include a sufficient number of samples that could reduce potential interference by rare polymorphisms in probe or primer binding sites. Assay validation should include adequate numbers of specimen types (B-I).

Rationale 1

If, for example, the test is to be performed on buccal swabs, whole blood and paraffin sections, then validation studies must be performed to determine the performance characteristics (ie, yield, size, purity, and removal of inhibitory substances) on each of these sample types. Extraction of nucleic acids should follow molecular pathology guidelines. However, no data is available at present to indicate a need to validate assay using set numbers of samples where epigenetic effects may differ in patient populations (eg, DNA methylation may differ for each sex and/or patient age). Controls that detect all possible variants may be used to confirm lot-to-lot differences and daily quality assurance. Synthetic DNA controls (including plasmids) may be used in this case. Alternatively, a subset of pooled specimens may be used to check the lot or daily runs in numerous runs if the use of pooled samples does not adversely affect the performance of the assay. Another alternative is to rotate controls so that there is quality assurance (QA) for each reportable allele. The QA for each variant should be performed in a timely fashion to permit retesting if that assay fails to pass appropriate quality assurance. The timeliness of QA testing depends on the clinical consequences of reporting an incorrect result. Whole genomic amplification of rare variants is permitted subsequent to validating the performance or quality of this material against non-amplified materials. This recommendation recognizes that extremely rare variants may not be amenable to this recommendation due to the scarcity of samples. Upon validation, the sample can be used within the laboratory in an alternative proficiency testing biannually (11-14).

Rationale 2

The quality of whole genomic amplification depends on the quality of the source nucleic acids. Poor quality reference DNA

can result in non-identical allelic representation for the whole genomic amplified samples. Therefore, the ability of the whole genomic amplified sample to function as a control must be determined prior to its incorporation into the assay (13).

Rationale 3

The use of controls to monitor and/or confirm test performance between lots and from assay to assay are required to identify failed assays in a timely manner. Timely identification of failed runs allows for corrective action and avoids delaying patient results (5).

e) Is proficiency for pharmacogenetic testing different from other molecular diagnostic tests and how often should it be performed?

Recommendation 6

Presently no evidence suggests that proficiency testing of PGx genotyping differs from other molecular diagnostic tests. Therefore, proficiency testing should be performed as required by the laboratory accrediting agency. This recommendation addresses alternative proficiency testing when assays are not included in proficiency programs (A-I).

Rationale 1

Ideally, proficiency should include whole genomic specimens that have been tested by another laboratory for the particular assay. In the case where no such proficiency samples are available, previously tested samples may be de-identified and a key to the de-identified samples retained. The key must not be released to the testing personnel until completion of the proficiency run. In this case, the assay can be validated using a reference method to initially validate the genotype (5).

Rationale 2

Proficiency testing is required to assure and improve the quality of laboratory testing (5, 15).

f) If software is used in interpretation, will it automatically flag extremely rare or unlikely allele combinations?

Recommendation 7

The software used in analytical instruments to generate a genotype should include methods or data analysis techniques needed to avoid inaccurate test results. The data reduction techniques used by the software to detect analytical errors should be disclosed to the end-user or an alternative service be provided to check the integrity of the data analysis (B-III).

Rationale

Software logic permits the laboratory to identify potential faulty results if the test generates a combination of genotypes from a patient which is statistically improbable. This tool will permit the laboratory to repeat the assay prior to reporting the result to the patient, thus assuring test quality and patient safety. Software commonly used in analytical platforms detects aberrant analytical results. Manual checks of the software calculations may be done periodically.

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Chapter **4**

Clinical Laboratory Services Considerations

Mark W. Linder and Werner Steimer

GENERAL INTRODUCTION

Providing clinical laboratory services is of central importance in establishing appropriate utilization of pharmacogenetic information to clinical practice. Thus, the laboratory must provide services consistent with the needs of health care providers. To provide these services in the context of pharmacogenetic testing, it is essential that, as with other clinical testing domains, laboratory operations and procedures be rigorously controlled. Because PGx is in the early stages of development as a field, it becomes important to utilize not only well-established and rigorous laboratory practice but also to consider processes that may be necessary to ensure adequate compliance that may be either unique or somewhat specific to the development of this new discipline, combining pharmacology and genetics in the context of laboratory medicine.

Questions for consideration are:

a) What level of certification should be required for clinical laboratories and personnel performing pharmacogenetics testing?

b) What are the recommended specimens for testing?

c) What should be the primary test-result output?

d) What criteria should be used to establish which genetic variants of a locus should be included for diagnostics purposes?

e) Is it necessary for there to be evidence to demonstrate cost effectiveness before recommending clinical use of PGx tests?

RECOMMENDATIONS

a. What level of certification should be required for clinical laboratories and personnel performing pharmacogenetics testing?

Recommendation 1
Whenever possible, and when required for laboratory compliance with local regulations, personnel performing pharmacogenetic testing in the United States must be qualified to perform "high complexity testing" as defined by CLIA Subpart A Sec 493.17(a) or as required by individual states. In other countries or municipalities, the appropriate accrediting agency will dictate appropriate regulations (A-I).

Rationale

In the United States, three categories of laboratory services have been established, including moderate and high complexity. A determination of testing complexity is made based on a cumulative score for seven criteria, each ranked as 1, 2, or 3, with 1 representing little complexity and 3 representing the highest complexity. Table 4 lists the scoring based on the consensus of the authors of this document.

Table 4. Tests having a cumulative score of > 12 are considered to be of high complexity (CLIA regulations subpart 493.17) (1).

Criteria	Rank
Scientific knowledge	3
Training and experience	3
Reagents and materials preparation	2-3
Characteristics of operational steps	2-3
Calibration, QC, and proficiency testing materials	3
Test system troubleshooting and maintenance	2-3
Interpretation and judgment	3
Cumulative score	18-21

b) What are the recommended specimens for testing?

Recommendation 2
The recommended specimen for testing is whole blood or a properly validated alternative sample (B-II).

Rationale

The quantity and quality of DNA isolated from whole blood specimens typically exceeds that obtained from alternative sources. However, service requirements, technical capabilities, and health care provider safety issues are increasing the demand for molecular diagnostics that do not require genomic DNA isolated from blood. The availability of techniques that can reliably use non-blood sources of genomic DNA have advantages in that such techniques avoid barriers to testing and enable collection of the specimen at the point of care when on site phlebotomy is not available. Due to variability in DNA quality obtained from alternative sources, assay reliability should be validated. Evidence for validation of alternative sources of DNA is the responsibility of the end user and may be provided based on assay manufacturers' data when available or through internal documentation.

c) What analytical information should be included with the test result?

Recommendation 3
The report should include sufficient information to establish the structural features of the tested gene and the results of that testing (A-I). The committee is cautious about recommending a specific format for reporting variants at this time.

Rationale

This recommendation is not intended to address the comprehensive question of pharmacogenetic test result reporting. That issue is dealt with in the next section in more detail. This recommendation is intended to ensure that the analytical aspects of the patient results are adequately documented for future reference and comparison between methods. It is not recommended that a phenotypic interpretation or allele designation (eg, CYP2D6*4) be reported in the absence of the structural finding. Despite efforts to standardize nomenclature systems for genetic variation, sufficient ambiguity remains and newly discovered features of previously defined alleles continue to antiquate their earlier designation. Further, not all assays for a given locus will include the same features. Thus it is recommended that all of the features tested (defined as objective findings (2) of the genetic locus be specifically identified. The features tested may be indicated by referencing the specific test kit used to perform the analysis provided a detailed list of the locus features included in the analysis are clearly defined within the manufacturers labeling. The committee is cautious about recommending a specific format at this time. This is being discussed for other inherited conditions, and a consensus is not yet reached. For up to date information regarding consensus nomenclature and formatting refer to Human Genome Organisation web site (3).

Final reporting and interpretation is covered in more detail in the Chapter V of this document.

d) What criteria should be used to establish which genetic variants of a locus should be included for diagnostics purposes?

Recommendation 4
PGx assays designed for clinical application should include genetic alterations of the target locus for which there is a well-defined influence on the function of the locus product or for which there is a clear relationship between the structural characteristic and observable influence on drug pharmacokinetics, pharmacodynamics, and/or toxicology (B-III).

Rationale

The key to this recommendation is the provision that there be a well-defined influence of that feature on a well-defined PK or PD end point. At this time, there is insufficient information and or guidance to establish criteria for inclusion of genetic variants based on population allele frequency.

Recommendation 5
NACB recommends that criteria be established to guide the selection of which genetic variants to include in diagnostic assays (A-III).

Rationale

The overall error for the test should be determined for each practice setting taking into account the differences in allele distributions and relevant phenotypes. Using CYP2D6 as an example, the *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, and *CYP2D6*6* alleles are reported to account for up to 98% of inactive alleles in Western European ancestry (or Caucasian) populations (4-6). Approximately 7% of the population is genotypic PMs thus the total frequency of poor metabolizer alleles must be 0.265. Given that *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, and *CYP2D6*6* collectively account for up to 98 % of poor metabolizer alleles, the cumulative frequency of the remaining alleles is approximately 0.006. Laboratories testing for only the most common four alleles will misidentify approximately one in 22 PMs as heterozygous for one correctly identified poor metabolizer allele and one allele misidentified as an active allele. Thus, the overall error would be one in 300 subjects tested. This is an example only and should be determined for each practice setting taking into account differences in allele distributions arising from differences in geographic genetic exchange. Laboratories should disclose what genetic features are included in their analysis and anticipated sensitivity and specificity of the techniques for discrimination of relevant phenotypes. Further, it is important to recognize that several alleles common in certain populations are associated with decreased activity, such as the *CYP2D6*10* and *CYP2D6*17* alleles. These alleles are known to reduce metabolic capacity and their clinical relevance is currently under investigation.

e) Is it necessary for there to be evidence to demonstrate cost effectiveness before recommending clinical use of PGx tests?

> ***Recommendation 6***
> Clinical laboratories should provide testing services when there is a convincing rationale for doing so. Criteria should be established to demonstrate this (A-III).

Rationale 1

Convincing reasons for diagnostic assays or procedures include evidence based on outcomes. Outcomes can be defined as: prediction of dose-related blood concentrations; mitigation of drug-related adverse events; improvement in response to therapy; reduction of overall cost of treatment and global economic impact (6). At the time of preparing this document, there is a lack of consensus and harmonization between regulatory as well as professional groups with regards to defining "convincing rationale". Many groups recognize convincing rationale as the ability to define a phenotypic characteristic such as drug clearance or estimated dosage, others hold convincing rationale to the standard of improved clinical outcomes within boundaries of cost effectiveness. This dichotomy must be addressed in a manner that limits inappropriate utilization and claims while not stifling innovation.

Rationale 2

Pharmacogenetic testing should be requested at the time of therapeutic intervention and is not currently recommended for general population screening.

Several criteria exist as rationale for providing a PGx test, one of which is FDA-guided labeling of a specific medication. One criteria the FDA has chosen is mitigation of risk, and in this context, the FDA has acknowledged the importance of testing for *CYP2C9* and *VKOR C1* genetic variation during the early phase of warfarin therapy. Further, the FDA has acknowledged the importance of testing for *TPMT*, for individuals to be treated with azathioprine. In addition, *UGT 1A1* genotype is acknowledged for patients to be treated with Irinotecan (7). These decisions were largely driven by the strong evidence to suggest that individuals who have these genetic variants are, in statistical terms, at significantly increased risk of a drug-related adverse event when these medications are administered. These acknowledgments were not based directly on determination of cost effectiveness or quality of life years, but on increased risk.

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Chapter **5**

Reporting and Interpretation of Pharmacogenetic Test Results

Jean-Pierre Morello and Roland Valdes Jr

GENERAL INTRODUCTION

When reporting genotype information from pharmacogenetic tests, the clinical laboratories must keep in mind that the end user of this information will most likely be the physician or other health care provider. It is usually the physician who will report the findings to the patient and take action using the test result. For the physician to correctly interpret the genotype information, it is beneficial that he or she have the complete diagnostic setting of the patient, including present and past drug regimens, medical history, and lifestyle. The following guidelines will detail several key recommendations and elements that should be included in the clinical laboratory and interpretive report.

Questions for consideration are:

a) What information should accompany the reported result?

b) Should the result be linked to a specific drug usage (as indicator)? Should drug "dosing and usage" information accompany the test result?

c) Should laboratories reporting PGx test results provide a consultation component or service available or by referral?

d) Should analytical limitation of the PGx test be indicated in the laboratory report?

e) Are there unique or specific limitations to be considered regarding confidential reporting of PGx test results?

RECOMMENDATIONS

a) What information should accompany the reported result?

Recommendation 1
Laboratories reporting pharmacogenetic genotype test results should be prepared to provide an educational resource to recipients of the test results to explain the complexity of the metabolic pathways involved; also be prepared to provide guidance as to which genes should be tested for a given clinical situation when known (A-II).

Rationale

Understanding drug metabolism pathways is central to interpretation of PGx tests. Thus, an appropriate consultation component is essential for optimum clinical application of the test results. Whether the physician is concerned about the effects of a drug he/she has previously prescribed and failed to offer clinical benefit (retrospective analysis) or is concerned about future benefit (prospective analysis), he/she will need to know the contribution of different CYPs to drug metabolism in order to correctly evaluate which cytochrome p450 gene will require genetic testing. For example, the commonly prescribed antidepressant citalopram is metabolized to N-desmethylcitalopram by CYP2C19 (1,2). N-desmethylcitalopram is then metabolized by CYP2D6 now, to N-didesmethylcitalopram (3-5). For citalopram, the antidepressant activity resides in the parent compound, and therefore, it is *CYP2C19* which will require genetic testing, even though CYP2D6 plays a role in the overall metabolism of citalopram.

b) Should the result be linked to a specific drug usage (as indicator)? Should drug "dosing and usage" information accompany the test result?

Recommendation 2
To provide optimum interpretive guidance relative to a specific drug or similar family of drug substrates, a laboratory providing PGx test results should be ableóif requested and whenever possibleóto provide information on drug substrates involved in the clinical situation (B-III).

Rationale 1

Associating genotype to clinical metabolizer status. Without scientifically validated data, it is dangerous to assign a phenotype to a genotype. This was very well illustrated for CYP2C9 where variant alleles (eg, *CYP2C9*2* and *CYP2C9*3*) are associated with diminished enzyme activity. Studies by Kirchheiner and colleagues (6) have shown that although individuals genotyped as either *CYP2C9 *2/*2* or *CYP2C9*3/*3* are classified as "poor metabolizers", reduction of drug clearance was highly variable for many drugs (6). This illustrates that overall phenotypic classification depends on both genotype and drug

(substrate). As we will see in the next section, drug-gene interactions also affect clinical phenotype.

Rationale 2

Providing patient-specific drug-gene interaction information. Given the expanding knowledge of drug/cytochrome p450 interactions, an ideal interpretive genotype report, for example, should optimally analyze the patient's current (and past) drug regimen and other parameters to evaluate the risk of drug/cytochrome p450 interactions. As an alternative, the report may include at least a few examples of drug/cytochrome p450 interactions that would caution the physician to consider these types of inhibiting interactions. For example, it is known that a number of antidepressants that function as selective serotonin reuptake inhibitors (SSRIs) can inhibit one or more cytochrome P450 enzymes and that this inhibition can be anywhere from mild to potent (7). Therefore, even though patients are genotyped as extensive metabolizers for a given cytochrome p450, if they are/were taking drugs that have cytochrome p450ñinhibitory activity, their actual enzyme activity can be as low as that found in poor metabolizers (8-13). Once the patient is no longer taking drugs that act as cytochrome p450 inhibitors, his/her metabolizer status will gradually return to that of an extensive metabolizer.

c) Should laboratories reporting PGx test results provide a consultation component or service available or by referral?

Recommendation 3
Laboratories providing PGx test results should be prepared to provide information as to interpretation of those results. Laboratories not equipped to provide test-interpretation capabilities as part of their test-reporting process should seek alternative mechanisms to provide those interpretive services in order to generate a more complete test report (B-II).

Rationale 1

These guidelines recognize that interpretive genotyping reports are complex and involve an integration of a number of factors that extend beyond the patient's genotype. Seeking the advice of organization or individuals, such as pharmacists, clinical pharmacologists, or toxicologists, offering these kinds of interpretive genotype reporting can serve as bridge between clinical laboratories and physicians.

Rationale 2

It is not advisable that clinical laboratories indicate a specific drug dosage for a patient. Many variables influence the dosage requirements for appropriate patient-specific therapy, most clinical laboratories will not have access to that information. However, in the context of appropriate and relevant clinical information specific to that patient and the drug in question, the laboratory may provide dosage information that is adequately supported by appropriate documentation.

d) Should analytical limitations of the PGx test be indicated in the laboratory report?

Recommendation 4
A report documenting a PGx test result should provide the recipient of the information sufficient detail documenting the analytical methodology used to obtain the result and address known limitations that may influence the robustness, interpretation, sensitivity, and specificity of the test result (A-I).

Rationale

The robustness and limitation of a PGx genotyping test results may be limited by the method used to perform the analysis. Ability to detect full-length gene duplication of alleles, assess their number, and be influenced by collection methods can all play a role in the robustness of the result thus place limitations on the interpretation (14,15). For example, the Tag-It kit from Tm Bioscience can detect that a duplicated allele is present but cannot determine in the heterozygous patient which allele is duplicated. The Roche Amplichip CYP450 system and long-range PCR-RFLP techniques (16) can determine which allele is duplicated in a heterozygous patient. Neither system identifies the number of additional gene copies that exist. Copy number (N), particularly in a heterozygous patient, can change the phenotype: *1xN/*4 would be an extensive metabolizer if N = 2, but a rapid metabolizer if N = 3 or higher. The intent of this recommendation is to relay information relevant to the analytical limitations of the genotyping method being used and is consistent with CAP requirements for molecular diagnostic tests.

e) Are there unique or specific limitations to be considered regarding confidential reporting of PGx test results?

Recommendation 5
A pharmacogenetic test result should be documented in a manner consistent with rules pursuant to routine demographics as required for CLIA compliance and protection of sensitive genetic information required by HIPAA, if in the United States, or as may be required in other countries (A-I).

Rationale 1

The geographic location of the testing laboratory, and ultimately the location of the recipient of the test information, dictates the rules that apply to the dissemination of the genotyping test results. Laboratories reporting PGx test results and the end users of the test results should comply with applicable restrictions. For example, a source might be using rules pursuant to HIPPA guidelines (17).

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Chapter **6**

Clinical Practice Considerations

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GENERAL INTRODUCTION

One of the most challenging aspects of transitioning the science of pharmacogenetics to the bedside is establishing criteria for its clinical application. Although this discipline is in its infancy, there are several examples that serve as fruitful models with which to establish guidelines and set future criteria for clinical implementation.

Pharmacogenetic tests have applications in a wide variety of drug-based therapies some of which are more well-established than others. In certain circumstances the genetic variability of an enzyme or other protein involved in the medication's metabolism or function may not constitute a clinically relevant variable as has been pointed out by the Duke-evidenced based Practice Center in regards to cytochrome P450 polymorphisms and selective serotonin re-uptake inhibitors. In contrast there are specific examples where the clinical relevance is much better defined (1). Our approach for these present practice guidelines is to take several key examples and use them as a basis for setting criteria to document future development of these services to medical practice. The term "practice" in this sense has a broader meaning than is usually ascribed. For example, a practitioner may be a physician or clinical pharmacist wanting to use PGx data to determine dosing or drug selection for a patient. A clinical researcher designing a study to determine the clinical efficacy of using pharmacogenetic information is also a practitioner with a different goal. A clinical laboratorian establishing a pharmacogenetics laboratory is also practicing clinical PGx when deciding which clinical scenarios to develop testing profiles for maximum clinical impact. Other situations apply as well to the definition of "practice", thus establishing a framework for building clinical practice guidelines for an infant discipline that depends on documenting several key examples from both the literature and personal experiences of practitioners.

As models, we consider six situations in which PGx test results have been reported to be useful for establishing criteria for clinical applications: azathioprine (oncology), warfarin (anticoagulation), atomoxetine (psychiatry), tamoxifen (oncology) and irinotecan (oncology), and abacavir (anti-retroviral). These six models, when combined, demonstrate several strategies and concepts for future development of PGx applications. Other pharmacogenetics applications exist, and the selection of these six examples is indicative of their acceptability or importance.

The metabolic response or immunologic characteristic of the patient revealed by DNA typing should not be considered a disease but an alternative environmental interaction to an exogenous agent, a drug therapy. The phenotype is triggered by exposure to the agent, but in its absence, does not convey overt pathology. Thus, DNA typing for drug safety and/or efficacy presents a new capability to diagnose eminently preventable conditions such as drug induced syndromes and therapeutic failures. As such, it should gain more awareness in modern health care. The prospective use of DNA typing poses the potential of individualized therapeutic management and advances personalized medicine.

Questions for consideration are:

a) Which are the most current variant alleles for *CYP2D6*, *CYP2C9*, and *CYP2C19* recommended for clinical use?

b) What set of criteria (characteristics) should be required of a PGx test to make it useful in a clinical laboratory setting?

c) What examples are available that can presently be used as models for application of PGx testing in clinical settings?

RECOMMENDATIONS

a) Which are the most current variant alleles for *CYP2D6*, *CYP2C9*, and *CYP2C19* recommended for clinical use?

> ***Recommendation 1***
> The following variant alleles are recommended when performing PGx genotyping for *CYP2D6, CYP2C9*, and *CYP2C19* (B-III).

Rationale

The alleles of most clinical significance are precisely those where the molecular nature of the polymorphism has well defined biochemical effects. These alleles (Table 5, adapted from Andersson (2) are generally recognized as a minimum number of features for these genes that must be tested in order to generate clinically applicable information. However, recommendations four and

Table 5. Basic Alleles of Clinical Reference for CYP2D6, CYP2C9, and CYP2C19 to Measure in All Population Groups

CYP2D6			*CYP2C9*			*CYP2C19*		
Variant	**Alleles**	**Activity**	**Variant**	**Alleles**	**Activity**	**Variant**	**Alleles**	**Activity**
Duplication of *1	*2xN	Ultra	430C>T	*2	Deficient	681G>A	*2	Null
Deletion	*5	Null	1075A>C	*3	Deficient	636G>A	*3	Null
1707T>del	*6	Null						
1846G>A	*4	Null						
2549A>del	*3	Null						

five in Chapter 4 of this guideline establish the need for more specific criteria regarding which alleles to include in diagnostic assays.

Table 5 is an example of allele listings, annotated with predicted biochemical effects at the protein level (2). Detection of null alleles leading to compromised metabolizer status is of primary clinical relevance. Additional genetic variation in each of the above enzymes has been reported and is likely to be identified as more individuals are genotyped. Recommendations for inclusion of alleles in diagnostic testing methods will need to be periodically re-evaluated as more data become available on molecular mutations. Population-specific alleles should be considered according to the ethnogeographic origin of the population served (3).

A particular variant is not always phenotype specific in that the variant may have a different impact depending on the drug (substrate) in question. For example, the classical literature on *CYP2D6*17* suggests that this allele tends to be associated with lower metabolic activity, but the decrease is not homogeneous among substrates. Studies using Risperidone (a psychoactive drug) suggests that *CYP2D6*17* is associated with normal metabolic capacity for Risperidone metabolism (4). This suggests that *CYP2D6*17* may influence various drugs differently as previously described (5). Similar substrate specific effects for CYP2C9 have been documented; for example, celecoxib metabolism is not altered in the case of the *CYP2C9*2* allele (6).

b) What set of criteria (characteristics) should be required of a PGx test to make it useful in a clinical laboratory setting?

Recommendation 2
For a PGx test to be useful in a clinical laboratory setting, it is recommended that the test have analytical and operational features that render them useful and compatible with clinical practice (B-II).

Rationale

The following are general such characteristics that apply to pharmacogenetic tests. *Analytical reliability:* the test should yield consistent measurement or detection of the desired analyte. *Operational implementation:* the test should have operational characteristics within the level of complexity certified by CLIA for clinical laboratories. *Clinical predictive value*: the interpretation of the test should have levels of clinical specificity and sensitivity consistent with the intended use of the test or examination. *Compatibility with therapeutic management:* the interpretation of the test could be useful for guiding therapeutic management and decision making.

c) What examples are available that can presently be used as models for application of PGx testing in clinical settings?

AZATHIOPRINE MODEL

Recommendation 3
TPMT genotyping is recommended as a useful adjunct to a regimen for prescribing azathioprine (A-I).

Rationale

Azathioprine and other mercaptopurine agents are used as therapeutics in several clinical situations, including, but not limited to, gastrointestinal inflammatory disorders and leukemias (7). These compounds are metabolized to inactive metabolites by several metabolic pathways. One major pathway involves the thiopurine methyl transferase (TPMT) enzyme (8). This enzyme is of particular significance because it is the sole pathway for mercaptopurine detoxification in erythropoetic tissues. Numerous studies have demonstrated that genetic deficiency identified through either genotyping or phenotyping is useful for the identification of high-risk individuals and can be applied to dose selection in order to manage safety (9). This particular application is a valuable example because extensive published work has provided proven alternative dosing strategies that accommodate the genotypic differences and allow for appropriate risk management without compromising efficacy (10).

WARFARIN MODEL

Recommendation 4
A combination of *CYP2C9* and *VKOR* C1 genotyping are the recommended PGx tests as adjuncts to individually adjusted dosages for Warfarin therapy (A-II).

Rationale

Warfarin is a frequently prescribed drug for both the treatment and prevention of thromboembolic complications. More than 21 million prescriptions are written annually in the United States for warfarin. Warfarin is a narrow therapeutic index medication with frequent complications despite dose adjustment for clinical variables, including age, sex, weight, nutritional factors, and interactive medications. Such complications range from occult bleeding to hemorrhage. NOTE: This application demonstrates the synergistic value of combining both pharmacokinetic and pharmacodynamic characteristics to arrive at a more meaningful approach to personalized medicine.

Warfarin is metabolized to inactive metabolites by the CYP2C9 subfamily of drug metabolizing enzymes (11-13). Approximately 25% to 35% of the population has *CYP2C9* alleles that lead to variably deficient enzyme activity and 3% to 4% of the population has 20% or less CYP2C9 drug-metabolizing activity (14,15). These variants can be detected by DNA analysis. These *CYP2C9* variants account for approximately 25% of the overall variability in warfarin dose (16-18). These variants lead not only to variable initial warfarin dose sensitivity but also to delays in achieving a stable maintenance dose, delays in hospital discharge, and increased bleeding complications (19).

Vitamin K Epoxide Reductase Complex protein 1 (*VKORC1*) is a characterized allelic abnormality in vitamin K metabolism (20). *VKORC1* is necessary to reduce oxidized vitamin K, which is required for post-translational maturation of the vitamin Kñdependent clotting factors II, VII, IX, and X. Genetic variants increase or decrease the responsiveness of this system and account approximately for an additional 25% of clinical variance in warfarin dosage. Because *CYP2C9* and *VKORC1* act independently, the total genomic-based warfarin variability is presently believed to be at least 50% (21). Decreased dose requirement of warfarin was originally associated with specific haplotypes of the *VKOR C1* locus (20). Subsequently, individual single nucleotide polymorphisms have been demonstrated to be directly associated with decreased dose requirement; these include the -1639 G>A and the 1173 C>T nucleotide substitutions (20,21).

Using multivariate regression models that include physical characteristics (such as age, sex, and weight) plus genotyping (*CYP2C9*2* and *CYP2C9*3* alleles, and *VKOR C1* -1639 G>A), several investigators have demonstrated superior estimation of warfarin maintenance dose when compared with use of either genetic factors or physical factors alone (21,23). It is likely that, as more data are generated, these multivariate models will also include quantitative influences of the *CYP2C9*5*, *CYP2C9*6*, and *CYP2C9*11* alleles as well as additional variants of *VKOR C1* such as the 3730 G>A and the 5417G>T [ie, (*4) (Asp36-Tyr)], which may be associated with higher daily dose requirements (eg, > 70 mg/wk) (24). Additional mechanism-based models are now being developed and validated as clinical decision support tools to enable long-term application of *CYP2C9* and *VKOR C1* genotyping testing results to more complex dosing scenarios, such as loading dose, transition dosing, and reconciling INR measurements with the status of achieving steady-state plasma S-warfarin concentrations (www.permitwarfarin.com (25)). Tools such as these establish a method for interventional application of PGx test results and are intended to support and educate clinical judgment.

TAMOXIFEN (NOLVADEX) MODEL

Recommendation 5
CYP2D6 genotyping may be useful as an adjunct to a regimen for prescribing tamoxifen (B-III).

Rationale

Tamoxifen, a selective estrogen receptor modulator, is widely used in the treatment and prevention of hormone-dependent breast cancer. Although it is a highly effective therapy, individual responses are not consistent. Studies indicate that part of this variability may be due to *CYP2D6*-mediated metabolism. For many years, it has been known that tamoxifen is extensively metabolised. However, only recently have studies unveiled a role for *CYP2D6* in tamoxifen metabolism and efficacy. *CYP2D6* appears to be the rate-limiting enzyme in the formation of the tamoxifen metabolite, endoxifen (4-hydroxy-N-desmethyl-tamoxifen (26).

When compared with the parent drug, endoxifen has a 30- to 100-fold greater anti-estrogenic potency (27,28). Although the potency of endoxifen is similar to another metabolite, 4-OH-Tam (4-hydroxy-tamoxifen), the average concentration of endoxifen is 6- to 10-fold higher (29,30). However, the range of endoxifen concentrations is also quite large. Part of this variability is due to genetic variability in *CYP2D6* activity. For example, endoxifen concentrations were approximately three-fold lower in patients who were deficient in *CYP2D6* activity (29,31). The *CYP2D6* deficiency can be a result of either inhibition by concurrent use of *CYP2D6* inhibitors, or by genetic variants in the *CYP2D6* gene.

Based on these findings, several studies have focused on the effect of the *CYP2D6* genetic variants on tamoxifen efficacy. One of these studies (32) a retrospective analysis of a prospective tamoxifen study, observed significantly worse outcomes in patients with genetic or drug-induced CYP2D6 deficiencies. They also noted that none of the CYP2D6-deficient patients who received tamoxifen developed severe hot flashes. A second study, which was in the breast cancer prevention study, also found worse outcomes in subjects with CYP2D6 deficiencies. The results of these studies are consistent with what was expected based on the metabolism and preclinical studies. However, contradicting results have been observed in other studies. Nowell et al (33) did not observe an effect of CYP2D6 on clinical outcomes. Also, research by Wegman et al (34) showed no association in one study and better outcomes in patients with CYP2D6 deficiencies in another study (35). The reasons underlying these inter-study differences are the focus of several ongoing studies. If the ongoing studies clarify the differences between these clinical outcome reports, *CYP2D6* genotyping may become incorporated into the decision-making algorithms to help personalize breast cancer endocrine therapies.

ATOMOXETINE (STRATTERA) MODEL

Recommendation 6
CYP2D6 genotyping may be useful as an adjunct to a regimen for prescribing atomoxetine (Strattera) (B-III).

Rationale

The close apposition of DNA typing and drug label recommendations and advisories present an immediate role for personalized medicine in modern health care. The product insert label for atomoxetine already has significant CYP2D6 guidelines. The label for atomoxetine, used in attention deficit hyperactivity disorder (ADHD) in children, adolescents, and young adults warns that poor metabolizers have plasma concentrations that are five times greater than observed when extensive metabolizers are administered the same dosage and, have an increase in half-life from 5 to 20 hours (refer to figure 3 Chapter 7). The label states "Laboratory tests are available to identify CYP2D6 poor metabolizers". CYP2D6 poor metabolizers cannot metabolize atomoxetine, and therefore, the dosage may be adjusted per guidelines in the product label. Further, the product label also indicates that some adverse reactions are more common among CYP2D6 poor metabolizers. Drug interactions with CYP2D6 inhibitors (paroxetine, fluoxetine) reduce atomoxetine metabolism to a similar extent as observed in CYP2D6 poor metabolizers. Assessment of dose reduction for children and adolescents may use the algorithm specified in the drug label for patients receiving CYP2D6 inhibitors.

	<70 kg body weight	**>70 kg body weight**
Extensive metabolizer	1.2 mg/kg/day	80 mg/day
Poor metabolizer	0.5 mg/kg/day	40 mg/day

These dosage adjustments pertain to poor metabolizer phenotype induced by drug interactions, and are not derived from genotype correlations. An article from the manufacturer, Eli Lilly (36) describes how *CYP2D6* affects drug levels but does not influence ADRs. Such data are thoroughly evaluated by the FDA, and the information on the drug label provides the standard prescriptive guidance from metabolizer status. Although some indications where this is not useful.

IRINOTECAN (CAMPTOSAR) MODEL

Recommendation 7
UGT1A1 genotyping is recommended as a useful adjunct for high-intensity irinotecan (Camptosar) dosing regimens (A-II).

Rationale

On August 22, 2005, the FDA approved a molecular assay (Invader UGT1A1, made by Third Wave Technologies Inc) for use in identifying patients that may be at increased risk of adverse reactions to the chemotherapy drug irinotecan HCl (Camptosar) used in the treatment of colorectal cancer. The test detects and identifies specific mutations in the gene that produces UDP-glucuronosyltransferase 1A1 (*UGT1A1*), an enzyme that conjugates the active metabolite of irinotecan (SN-38) to form a glucuronide metabolite. Clinical studies have shown the assay to be 100% accurate compared with DNA sequencing, the standard for genotype determination (n = 285, 95% lower limit on confidence = 99%). *UGT1A1* activity is reduced in individuals with polymorphisms of the *UGT1A1*28* allele, which is homozygous in approximately 10% of the North American population. In a prospective study of 66 patients administered high-intensity irinotecan therapy, the mutation was associated with a 5-fold increase in the risk of drug-related toxicity related to increased blood levels (37).

According to updates in the safety labeling for irinotecan, a one-level reduction in initial high-intensity (ie, 125 mg/m^2) irinotecan dose should be considered in patients known to be homozygous for the *UGT1A1*28* allele. Because the precise dose reduction in this patient population is not known, subsequent dose modifications should be considered based on individual patient tolerance to treatment. The FDA noted that the assay is intended for use as an aid in making individualized patient treatment decisions and is not a substitute for a physician's judgment and clinical experience. Other important factors such as liver and kidney function, age, and co-administered drugs should also be considered. The utility of *UGT1A1* genotyping in the context advanced multi-drug regimens is not addressed in the product label.

ABACAVIR MODEL

Recommendation 8
Genotyping for human lymphocyte *HLA-B*5701* is recommended prior to administering abacavir for patients who are infected with the human immunodeficiency virus (HIV), in order to avoid the development of a delayed hypersensitivity reaction (A-II).

Rationale

Abacavir is an anti-retroviral agent used to treat patients infected with HIV type 1. Approximately 5% to 8% of patients on abacavir experience a hypersensitivity reaction within 6 weeks of initial therapy. The manifestations of hypersensitivity reaction range from fever and rash to life-threatening fulminant hepatic and renal failure (38). The presence of the *HLA-B*5701* allele predisposes an individual to hypersensitivity reaction. The allele frequency is highest among the Caucasian population at about 8% (39). One study found that an individual with this genotype has a 960-fold higher risk for development of hypersensitivity than an individual with the wild-type (40). On December 1, 2007, the Department of Health and Human Services Panel on Antiretroviral Guidelines recommended screening be conducted for the *HLA-B*5701* prior to starting patients

for abacavir therapy (41). Those with the *HLA-B*5701* allele should not be given this drug. These recommendations were based in part on the results of a double-blinded prospective randomized trial involving 1,956 patients from 19 countries (42). The study found the prevalence of the *HLA-B*5701* allele of 5.6%. There were no cases of immunologically confirmed hypersensitivity reaction in patients who were screened negative for the *HLA-B*5701* allele resulting in a 100% negative predictive value. The rate of hypersensitivity reaction for the patients who were randomized to the non-screening arm had a 2.7 incidence. Another study of an ethnically mixed French HIV population also showed no cases of hypersensitivity after a screening program was instituted (43). There are no commercial assays currently available for *HLA-B*5701* genotyping. However, the high degree of disease penetrance with abavacir pharmacogenetics coupled with release of national recommendations will prompt the in vitro diagnostics industry to begin work on making this test available.

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Chapter 7

Therapeutic Drug Monitoring and Pharmacogenetics Interface Considerations

Les Shaw and Gil Burckhart

GENERAL INTRODUCTION

One of the major roles of laboratory medicine and clinical laboratories in support of pharmacotherapy has been in the area of therapeutic drug monitoring (TDM) (1). Medications which require monitoring of their concentrations in blood are those where narrow therapeutic concentration ranges in blood are required for efficacy (eg, digitalis, some aminoglycosides, tacrolimus, methotrexate, lithium), and where toxicity is a persistent problem. As the discipline of pharmacogenetics (PGx) finds its way into clinical practice, the combination of traditional TDM and PGx must be explored to achieve optimum utilization of the combined information they provide. In essence, PGx provides information that allows the clinician to make a determination of appropriateness and risk of drug therapy prior to the initiation of therapy. PGx may then have an additional place in selecting drug or dosage alterations during the treatment for a disease process.

The evaluation process in TDM is essentially a phenotyping procedure that globally reflects the status of the patient's disease, or represents the pharmacokinetics and pharmacodynamics of a drug or drug regimen. Pharmacokinetics are evaluated by the change in concentration over time while pharmacodynamics are evaluated by the relationship between concentration and effect. Because TDM is not just drug levels alone, the broader field of PGx may also play a role in TDM in the future. The combination of PGx and TDM can be powerful because each complements the other, as demonstrated below.

Questions for consideration are:

a) How should standard TDM practices be modified to account for pharmacogenetic variation?

b) Are there specific clinical situations demonstrative of both TDM and PGx information having complementary value?

c) How can TDM be best utilized in establishing the predictive value of PGx tests, as end point?

RECOMMENDATIONS

a) How should standard TDM practices be modified to account for pharmacogenetic variation?

> ***Recommendation 1***
> When the pharmacogenetic characteristic results in a change in drug pharmacokinetics or receptor sensitivity, TDM practices should take into account the necessary changes over time to reach steady-state plasma concentrations and drug clearance, as well as the need to adjust the typical therapeutic range for a drug according to the genotype (B-II).

Rationale

In order to properly evaluate the plasma-drug concentration that is established by a dosing schedule, the patient must have achieved a steady-state plasma drug concentration (2). Most standardized therapeutic drug monitoring protocols are designed to collect specimens once patients are assumed to have reached steady state, which is the time equal to approximately five elimination half-lives of the medication. Individuals with a genetic variant that decreases or increases the elimination half-life of the medications will also have an increased or decreased time to steady state (3). Therefore, standard TDM practices must be modified to account for this change in time.

Similarly, when the medication receptor abundance or affinity for the medication is altered as a consequence of genetic variation, the plasma drug concentration required to elicit the desired therapeutic response may have to be adjusted to concentrations outside of the typical therapeutic range. For example, standard practice of initial dose adjustments for the anticonvulsant phenytoin call for an initial measurement of phenytoin-plasma concentration on the third day of therapy. This practice is based on the fact that the average half-life of phenytoin is < 14 hours and therefore the patient is expected to be nearing a steady-state plasma concentration for that dose within about 72 hours. In contrast, an individual with a genetic deficiency in CYP2C9 will have decreased phenytoin

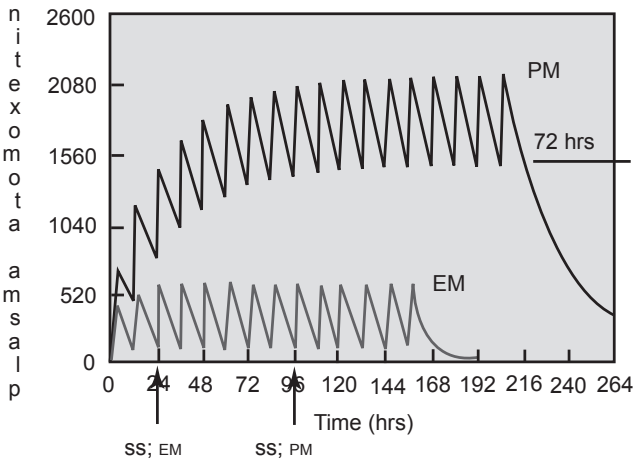

Figure 8. Atomoxetine plasma accumulation models for CYP2D6 extensive metabolizer versus poor metabolizer subjects. Models are based on 20 mg twice per day dosing and published pharmacokinetic parameters for both genotypes. Data taken from Sauer (4).

clearance (5) with an increased elimination half-life of more than 30 hours and therefore the plasma concentration measured on the third day of therapy will be much lower than the steady state concentration achieved after about 6 days of dosing.

As another example, atomoxetine elimination is about 4 hours in subjects who are CYP2D6 extensive metabolizers (6). As is true for drug accumulation to steady state, drug wash-out is also directly linked to the elimination half-life of the drug. There-fore, steady-state plasma concentrations will be achieved within the first day of therapy and upon discontinuation of atomoxetine, the atomoxetine is expected to be more than 95% eliminated within 24 hours of discontinuation, when an alternative therapy can safely be initiated. In contrast, a CYP2D6 poor metabolizer where the atomoxetine is also given standard atomoxetine dos-ages, steady-state plasma concentrations are not achieved until approximately 4 days of therapy. When achieved, they are about three times higher than anticipated for extensive metabolizer (4). Furthermore, upon discontinuation, plasma concentration is still well above expected peak concentration for EMs at 24 hours and does not return to baseline until 3 days after discontinuation.

An example of the potential necessity to change the therapeu-tic range for a given genotype is the reported genetic variation in the vitamin K epoxide reductase complex 1 protein. This protein has shown that the variant has no effect on warfarin clearance, but lower dosages are adequate to establish the target level of anti-coagulation. This is due to the fact that lower plasma concentration of the S-warfarin enantiomer is adequate to inhibit this complex most likely (7) because of a lower abundance of the protein.

b) How can TDM and PGx information provide complementary value?

Recommendation 2
It is recommended that PGx test information be used to support the selection of drugs or doses for drugs for which correlations between drug dosing and genotyping has been established. Traditional TDM practices should be employed to confirm and/or refine optimum dosing parameters (B-III).

Rationale 1

Statistically significant associations with genetic polymorphisms inherently have extreme variability in relation to many patient outcome parameters. The best documented of those parameters are between genetic polymorphisms and drug pharmacokinetics. Two examples of this include: tacrolimus in organ transplantation (8) and warfarin dosing (3).

Rationale 2

Figure 10 demonstrates that the application of multivariate regression models based in part on PGx information can provide and estimate of the optimal warfarin maintenance dose (7). However, traditional TDM using INR monitoring is still an essential component to the overall drug therapy.

c) How can TDM be best utilized in establishing the predictive value of PGx tests, as end point?

Recommendation 3
Plasma drug concentration measurements should be considered as an integral part of studies to characterize genotype-phenotype correlations (B-II).

Rationale 1

Plasma drug concentrations are valuable assets for evaluating pharmacogenetic variables. In the case of pharmacogenetic variants that influence drug pharmacokinetics, these measurements can facilitate the estimation of fundamental pharmacokinetic parameters (6). Pharmacokinetic parameters can facilitate genotype-guided dosing strategies to achieve normalized drug exposure between genotypes and allow for design of more appropriate monitoring strategies. This is arguably a valuable clinical end point as there are clearly associations between excessive plasma drug concentration and increased risk of adverse drug reactions (warfarin as an example). Modification of dosing practices to achieve plasma drug concentrations that

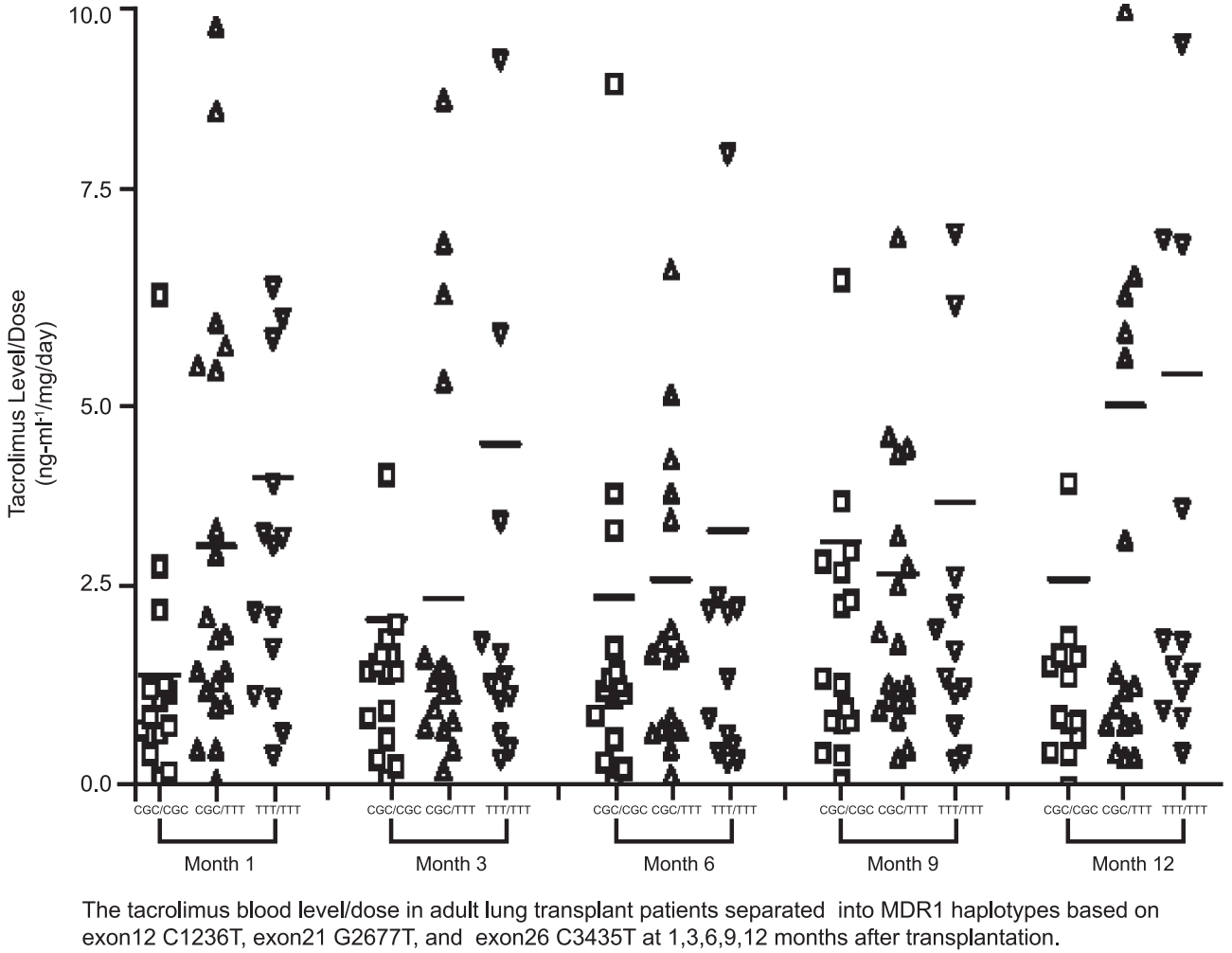

Figure 9. The effect of *ABCB1* haplotypes (CGC/CGC, CGC/TTT, and TTT/TTT) on tacrolimus level/dose in patients who have received lung transplant during the first year after transplantation. The horizontal lines represent the mean tacrolimus level/dose and a significant relationship does exist between *ABCB1* haplotype and tacrolimus level/dose during the first postoperative year (8). However, the raw data is presented to demonstrate that the mean data cannot be used to predict the tacrolimus level/dose on any individual patient.

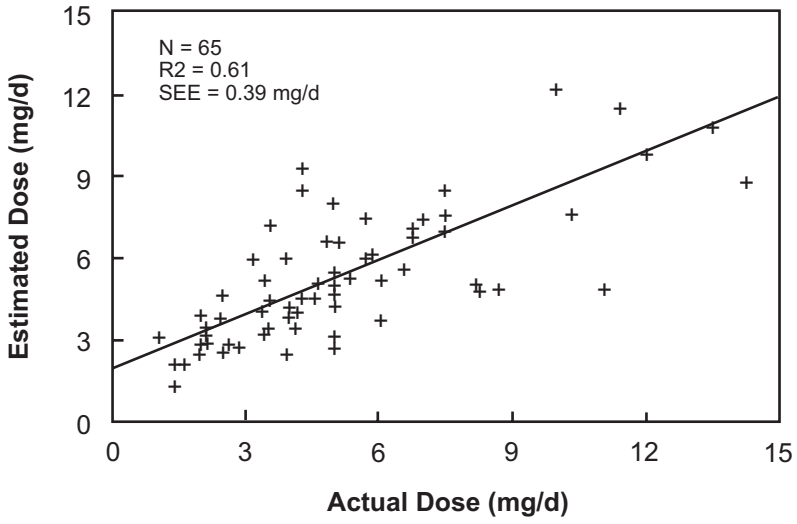

Figure 10. Correlations between estimated maintenance dose of warfarin and actual maintenance dose based on the model of Zhu et al (7).

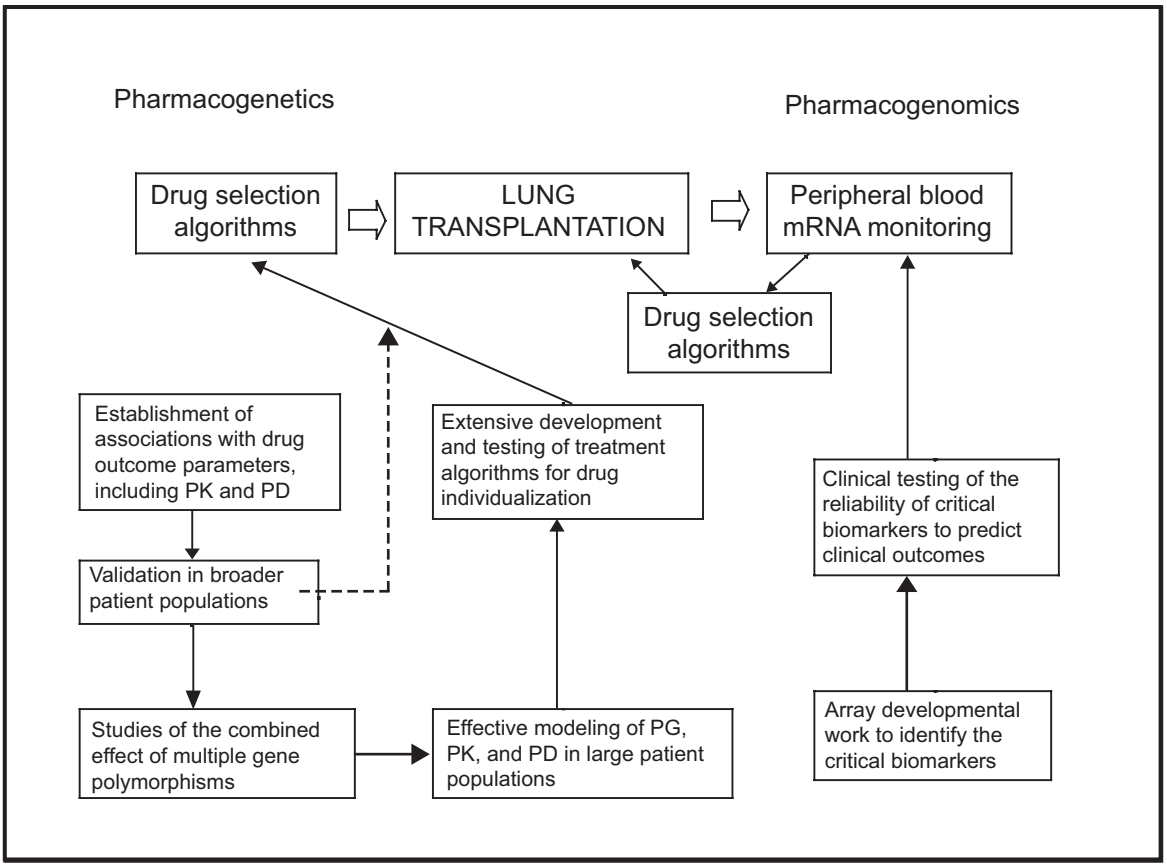

Figure 11. Graphic depiction of the steps involved with the development of pharmacogenetics and pharmacogenomics in patients who received lung transplants. The left side indicates the route for development of pharmacogenetic associations, and the route may be shortcut at the point of the dashed line for polymorphisms that are heavily weighted for impact on patient outcomes. The right side demonstrates the development of pharmacogenomic monitoring in lung transplant patients. Both sides come together in the treatment and monitoring of the lung transplant patient (9).

have been established as safe and effective for EMs is an excellent approach to deriving the much needed genotype guided dosing strategies and for estimating the improvement of pharmacogenetics in drug safety.

Pharmacodynamic variables may also be better defined by studies that incorporate plasma drug concentration measurements. For example, what is the basis for the association between the -1639 G>A promoter polymorphism of VKOR and decreased warfarin dose requirements? It has been hypothesized that this variant decreases *VKORC1* transcription and therefore limits the production of this protein. However, there are conflicting reports on this issue that had investigators who first reported on this genetic variant measured the steady-state S- and R- warfarin concentrations, it is likely that they would have confirmed the suspicion that the lower dose requirements are consistent with lower effective plasma concentrations in subjects with the -1639 AA *VKORC1* genotype as later demonstrated by Zhu et al (7). Likewise, if earlier studies to establish the role of CYP2C9 on warfarin safety had included plasma warfarin concentrations in all studies, the fact that some patients required lower dosages and had lower plasma drug concentration may have accelerated the discovery of a pharmacodynamic basis for low warfarin dose requirements.

Rationale 2

Given the variability supported in Recommendation 2, then it is logical to assess the outcome after drug or dosage selection is performed. This assessment may be made through TDM procedures including drug levels, but may also be made in the future using PGx assessment tools such as gene profiling in peripheral blood. Additional support for the need of assessing outcome after drug selection and administration comes from the fact that no work has been performed with PGx and drug and food interactions (9). Therefore, the magnitude of drug interactions cannot be predicted based upon PGx, and therefore TDM, including PGx testing, is necessary to measure outcomes and to reassess therapy (10). An example of application of TDM in the development of pharmacogentic approaches for guiding drug therapy is shown in Figure 11.

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Chapter **8**.

Ancillary Applications: Drug Prescription/Dispensing and Forensics

Saeed Jortani and Steven Wong

GENERAL INTRODUCTION

Pharmacogenomics has been focused on enabling the targeted practice of drug selection by health care providers. However, other applications of PGx are evolving beyond that domain, such as applications in production of targeted drugs by pharmaceutical companies, forensics, safer distribution of medications by pharmacist, environmental toxicology, predicting addiction to substances, and multiple other applications. The information is still much too fresh and rapidly evolving in many of these areas, so they cannot be considered for specific laboratory medicine application guidelines at this time. However, in the areas of forensics and drug dispensing, some recommendations can improve and enhance applications in the prescription of drugs and in forensics.

APPLICATIONS IN DISPENSING OF MEDICATIONS

Questions for consideration are:

a) Should information related to PGx test availability be made part of the information provided to patients when dispensing drugs? If so, by whom or how?

b) Should PGx test information be considered an integral part of drug-dispensing safety awareness?

c) Should information-related relationships be fostered between drug dispensing providers and clinical laboratories providing PGx testing services?

RECOMMENDATIONS

a) Should information related to PGx test availability be made part of the information provided to patients when dispensing drugs? If so, by whom or how?

b) Should PGx test information me considered an integral part of the drug-dispensing safety awareness practice?

Recommendation 1
After appropriate consent from the patient and establishing measures to protect his/her health information, PGx genotype information can be made available to drug-dispensing organizations to be used as part of their drug-dispensing safety verification procedures (B-II).

Rationale 1

Two areas of importance relative to application of PGx information provided by clinical laboratories (and likely useful to pharmacists) are the availability of genotype information for purposes of alerting either physicians or patients of possible drug interactions and linking that information to dispensing either at remote locations or in hospital settings. Relating information on drug-drug interaction has become routine in safely dispensing medications. The addition of genotyping information able to identify slow or rapid metabolizers (and eventually receptor-related genotyping) may assist the pharmacists in cross-checking the efficacy of the drug type ordered as well as the appropriate dosage and possible interaction with other concurrent medications that may be concentration dependent. The clinician clearly is the final decision maker regarding dosing and appropriateness of prescribed drugs. Laboratorians and pharmacists are involved in assuring patient safety by the use of pharmacogenetic information.

c) Should information-related relationships be fostered between drug dispensing providers at hospitals and clinical laboratories providing PGx testing services?

Recommendation 2
Hospital-based drug dispensing departments and clinical laboratories are encouraged to work in close collaboration and establish policies to make available timely genotyping information useful for guiding the dispensing of medication for hospitalized patients and for recommendations after discharge (B-II).

Rationale

Presently, acquiring clinical laboratory results by therapeutic drug monitoring and measuring other biochemical physiological

parameters (eg, creatinine, renal function) provide pharmacokinetic information to pharmacy departments and thus assist in optimizing drug administration. This typically requires close communication between the clinical laboratories and the dispensing units. Therefore, a close association between the clinical laboratory and the drug-dispensing entity at hospitals and clinics should be fostered and policies be established to encourage and permit this cooperation.

APPLICATIONS IN FORENSICS

The clinical use of genetic testing is gaining popularity in such areas as establishing proper drug dose requirements and evaluating potential drug toxicity to various therapeutics, such as the anticoagulants, antidepressants, antipsychotics, and pain management (eg, opioid drugs; 1-3). These groups of drugs are often identified as leading causes of death. Other areas of forensic toxicology, such as human performance or workplace testing, may also involve situations in which genotyping has potential value (4,5). In light of this, practitioners in forensic toxicology investigations have also considered the use of genetic testing in interpreting drug poisoning cases (6-9). Considering the fact that information gained through pharmacogenetic testing on a deceased individual may also impact the surviving family, issues regarding confidentiality and health information should be considered by the laboratories. In order to ensure sampling, testing and interpretive considerations in forensic implementation of such tests, we propose the following questions and recommendations.

Questions for consideration are:

a) In forensic applications of PGx testing, what is (are) the preferred specimen(s), and what diligence should be established for purposes of evidence acquisition?

b) What type of information and correlations should be used to optimize the application of PGx data in forensic cases?

c) What qualifications by way of training and experience should be required for individuals reporting and interpreting PGx information when applied to forensics?

d) What type of information should accompany a PGx test report as it applies to applications in forensics?

e) Are there any particular or specific ethical considerations that may apply to the use of PGx data with regard to applications in forensics?

RECOMMENDATIONS

a) In forensic applications of PGx-testing, what is (are) the preferred specimen(s), and what diligence should be established for purposes of evidence acquisition?

Recommendation 1
For forensic purposes, blood is considered to be the preferred specimen of choice and can be used whenever available (A-II).

Rationale

Many commercial pharmacogenetic tests have been validated for whole blood (10,11). Other samples, such as tissues, buccal swabs, and saliva are also being used to a lesser extent (12,13). Alternative tissue samples may be used if they have been shown to yield the required DNA amount essential for testing. Extraction, amplification, and validity of testing alternative specimens should have been previously established before their use in testing forensic samples (1,14).

Recommendation 2
Chain of custody should be maintained for forensic samples, according to the established protocols by each laboratory (A-II).

Rationale

As required by legal proceedings, such samples should be stored at 4°C in a locked cabinet until testing. The extracted and amplified genomic DNA should also be stored at -70°C for a minimum of 2 years after testing (1,3).

b) What type of information and correlations should be used to optimize the application of PGx data in forensic cases?

Recommendation 3
Whenever possible, in cases in which polymorphic enzymes are suspected as factors in drug toxicity, other relevant issues, such as polymorphisms in receptors, transport proteins, genes that affect pharmacodynamics should also be considered (B-III).

Rationale

If drug poisoning is indicated by case history and/or autopsy, measurement of blood and tissue concentrations of drug and its metabolites is routinely performed (9,14,15). After case review of drug toxicity involving polymorphic enzymes, genotyping for these particular enzymes and proteins is recommended. The latter will assist, as an adjunct, in interpreting the cause of overdosage and is not be used on its own to establish cause of death (1-3,16).

c) What qualifications by way of training and experience should be required for individuals reporting and interpreting PGx information when applied to forensics?

Recommendation 4
Interpreting pharmacogenetic testing results in forensic toxicology should be done by individuals with adequate training in forensics, toxicology, and pharmacogenetic testing and familiarity with metabolic pathways (B-III).

Rationale

Drugs and poisons may cause overdoses, chronic toxicity, or performance impairments, leading to accidents and fatalities. Determining the extent to which a drug contributed to a death requires correlating all aspects of the death investigation. These include the autopsy report, which generally includes toxicology findings, antecedent events, medical and social history, and thorough scene investigation (17,18). The pharmacogenetic data will therefore constitute only one piece of this entire puzzle and can be incorporated by individuals who are experienced in investigating drug-related deaths (3,14).

d) What type of information should accompany a PGx test report as it applies to applications in forensics?

Recommendation 5
Whenever possible, reporting should be accompanied by information about the degree of a particular polymorphism's role in the pharmacokinetics or dynamics of the drug(s) in question (B-II).

Rationale

Frequently, a given drug is metabolised by more than just a single enzyme. For example, methadone has been shown to be demethylated to its primary metabolite EDDP mainly by CYP3A4. Other enzymes, such as CYP2C9 and CYP2C19, also partially contribute to the metabolism of methadone to EDDP (19). Therefore, in interpreting a given methadone case, it would seem rational to take into account the contributions of CYP3A4, CYP2C19, and CYP2C9 to its metabolism. Furthermore, methadone has been shown to inhibit CYP2D6 activity, leading to interaction with drugs that rely on this route for metabolism by reducing their clearance (20,21). Another example is fluoxetine, which is metabolized by CYP2D6 and CYP2C9 (22).

e) Are there any particular or specific ethical considerations that may apply to the use of PGx data with regard to applications in forensics?

Recommendation 6
Ethical considerationócurrently, institutional review board and informed consent of the decedent's familyódo not enter in postmortem analysis. However, consultations with the supervising legal authorities/medical management is essential in maintaining high ethical standards and preserving the rights of the decedent and the family members (B-II).

Rationale

Pharmacogenetic testing and its applications to various clinical or forensic investigations, due to its heritable nature of DNA, raise several complex ethical, legal, social, and economic issues (23). Where there are no frameworks, it may be necessary to establish clear policies on pharmacogenetic issues. Many states already grant their medical examiners/coroners the authority to order testing, which would assist them in death certification (24). The ethical issue in such investigations stems from the possibility, remote though it may be, to extrapolate or infer pharmacogenetic information about a decedent's relatives from a decedent's sample. To protect the health information of the living relatives, it is advisable to obtain their consent prior to pharmacogenetic testing of the deceased. This practice would assure a high standard of ethics. However, many ethical issues around pharmacogenetic testing still remain (25).

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Chapter **9**

Regulatory Considerations

Felix Frueh and Atiqur Rahman

GENERAL INTRODUCTION

Regulatory considerations for the evaluating and approving pharmacogenetic tests can be grouped into two broad categories: analytical validation and clinical usefulness of the test. This evaluation hinges on the mission the FDA fulfills, namely, to advance public health by helping to speed innovations that make medicines more effective and safe.

Although tests are evaluated by the Center for Device and Radiological Health (CDRH) and drugs/biologics by the Center for Drug Evaluation and Research (CDER), the two centers interact closely, and they often jointly evaluate pharmacogenetic tests, particularly in cases where drugs and tests are co-developed and cross reference is being made in drug and device labels.

Considering the scientific evidence submitted for regulatory approval, the FDA creates, in cooperation with the applicant, a label (package insert) that describes the analytical and clinical characteristics of the product (ie, a drug or a device). For example, a label for a drug that should only be taken by a specific subpopulation describes the scientific evidence on why this drug is indicated for this population (ie, efficacy) and/or why it is not indicated for other populations (ie, safety and/or efficacy). In addition, the label provides information about the marker for which a test is indicated and, if available, the test itself. Together, this information allows the health care professionals and the consumer to make better decisions about treatment, helping to answer questions such as "will this drug work?" or "what is the best dose of this drug?" (1). Hence, the drug labels reflect a manual for the drug or device, in which information is provided about how best to use the product, based on the science associated with the product (2-4).

A survey of pharmacogenetic information contained in drug labels approved in the United States from 1945 to the present showed that, 121 of 1,200 such labels contained pharmacogenetic information (5). Not surprisingly, there has been an exponential increase in these labels in the last 15 years. Between 2000 and 2005, 43 new drug labels were approved that contained pharmacogenomic information, reflecting 37 percent of all new approved labels during that time period. The survey showed that the labels contained mostly pharmacokinetic (eg, drug metabolizing enzymes) data, which has relevance for drug dosing and adverse events. Newer labels also contained pharmacodynamic information (eg, receptors), which has relevance for the identification of responders, non-responders.

Broadly speaking, label information can be categorized into three general groups: labels in which pharmacogenetic tests are required (4; eg, Herceptin, Erbitux); labels in which pharmacogenomic test are recommended (3; eg, Irinotecan, 6-MP), and labels which contain only information about pharmacogenetics, but no reference is made as to whether a test is required or recommended (3; eg, Tarceva, Strattera).

Even though a significant number of labels for drugs and devices contain pharmacogenetic or pharmacogenomic information, this knowledge is often new and evolving. Consequently, and as it is the case for other, non-genomic information, it is not always possible strictly to require certain actions based on current genetic or genomic information. For example, even though there is scientific evidence indicating that erlotinib is less beneficial in epidermal growth factor receptor (*EGFR)*-negative patients, the current label for Tarceva® does not require this test in order for the drug to be prescribed and the drug is available to everybody (3). In contrast, the Her2/neu antibody trastuzumab has shown to be efficacious in *Her2/neu*-positive breast cancers and was studied only in this population, which allowed for a more restricted label pointing out that a positive human epidermal growth factor receptor-2 (*Her2/neu)* test is required before Herceptin® can be prescribed (4). To accommodate this variation in scientific evidence, the FDA considers the use of genomic information in drug labels either to require a genetic test for prescribing a drug, to recommend the use of a genetic test prior to drug therapy, or simply to provide information about the current knowledge of genomics that is relevant to drug therapy without the requirement or recommendation of a specific action.

Regulatory considerations for the evaluating and approving pharmacogenetic tests stem from the basic regulatory requirements for safety and effectiveness. For diagnostic use, a manufacturer must demonstrate that the label is consistent with valid scientific evidence and comports to the analytical and clinical performance of the test for its intended use. The diagnostic use of a pharmacogenetic test, by its very nature, is tied to therapeutic decisions, such as drug selection and dosing, and a test with insufficient validation data, even with known clinical utility, may result in incorrect therapy. For this reason, the FDA holds such tests to high standards of performance in its efforts to protect the public health.

Clinical utility is a pharmacogenetic test performance parameter directly linked with therapeutic outcome, and as such is typically specific to a particular drug or class of drugs. When clinical utility is more broadly applicable, for example, across classes of drugs, and the mechanism is understood, test performance can often be addressed at least partially by citing relevant peer-reviewed literature, so that existing and rapidly evolving knowledge may be taken into account. FDA also has allowed test labeling for certain pharmacogenetic tests that define genotype/phenotype correlations across broad drug spectra to be non-therapy specific, so that as new marker-drug interaction data evolves, existing tests may be applied to new drugs for which the marker is associated in a relevant and interpretable way.

NOTE. Following are questions, language, and recommendations formulated based on FDA policies at the time of writing. These five recommendations are interpretations of FDA guidelines that may be relative to FDA testing.

Questions for consideration are:

a) When will a test be "recommended" versus "required" for a clinical decision?

b) What type of information should be included in the label of a drug or test (eg, sensitivity, specificity, positive and negative predictive value, genetic background information, pharmacoepidemiologic information)?

c) What levels of performance are required before a test can be considered for use in a drug label?

d) How should test and drug studies be linked in order to gain drug/test colabeling?

RECOMMENDATIONS

a) When will a test be "recommended" versus "required" for a clinical decision?

Recommendation 1
A test may be required for a therapy when the drug or the biologic is co-developed with a test. Patients are eligible to receive a treatment only if a test result is obtained prior to treatment initiation (A-I).

Rationale

Certain targeted therapies may benefit only a population that shows a response marker (4). Testing for HER2/neu protein overexpression is required prior to the use of trastuzumab alone or in combination with other agents for metastatic breast cancer. Trastuzumab is a recombinant DNA-derived monoclonal antibody that selectively binds to the extra domain of HER2. Clinical trials for approval of trastuzumab were conducted in patients whose tumors overexpressed the HER2 protein, and clinical benefit of trastuzumab over other chemotherapy was established only in HER2 overexpressed population. Similarly, cetuximab was indicated for the treatment of patients with human EGFR expressing metastatic colorectal cancer. Patients enrolled in the clinical trials were required to have immunohistochemical evidence of positive EGFR expression in their primary tumor or tumor from a metastatic site. Specimens were scored based on the percentage of cells expressing EGFR and the intensity of expression.

Recommendation 2
The test may be recommended prior to the selection of a therapy and/or the selection of a dose for a particular population deficient in activity of a polymorphic enzyme involved in the inactivation of the drug/biologics (B-II).

Rationale

Toxicity of a drug/biologics may be associated with the inability of the body to inactive the agent through metabolism (2). Irinotecan is indicated for the treatment of metastatic colorectal cancer. Initial irinotecan dose reduction is recommended for patients who are deficient in uridine diphosphate-glucuronosyl transferase 1A1 (UGT1A1), the enzyme required for inactivation of the active metabolite of irinotecan, SN-38. Patients deficient in UGT1A1 activity are at increased risk for severe neutropenia resulting in hospitalization, dose reduction, and/or treatment delay. Testing for thiopurine methyltransferase (TPMT) status prior to or during 6-mercaptopurine or azathioprine treatment is suggested to avoid bone marrow related severe toxicity. Patients with two non-functional alleles have low or absent TPMT activity resulting in accumulation of toxic metabolites causing life-threatening myelotoxicity if they receive normal dose of 6-mercaptopurine or azathioprine.

b) What type of pharmacogenetic-information should be included in the label of a drug or test (eg, sensitivity, specificity, positive and negative predictive value, genetic background information, pharmacoepidemiologic information, test availability)?

Recommendation 3
The availability of a test may be mentioned in the label of a drug/biologics to inform the patients and the health care provider about the availability of the test (B-II).

Rationale

Information about the availability of a test to detect the genetic defect of an enzyme associated with the deactivation of the drug/biologics may be included in the label when a drug/biologics has a broad therapeutic window and a normal dose does not compromise with the safety and effectiveness of the agent (6). Atomoxetine is predominantly metabolized by CYP2D6. The drug is titrated to a maximum dose of 1.2 mg/kg and has a wide therapeutic window. Low starting dose allows for a safe dose titration to a desirable therapeutic effect in patients with CYP2D6 deficiency. The laboratory test section of the atomoxetine drug label includes information about the availability of laboratory tests to

detect the poor metabolizers of CYP2D6. Because the clinical impact of testing for dosing this drug for poor metabolizer has not established, inclusion of the test in the package insert is for information purpose only.

c) What levels of performance are required before a test can be considered for use in a drug label?

Recommendation 4
Pharmacogenetic tests should be adequately validated for their intended use. Additional safety and effectiveness concerns are linked to the level of risk of using the test results to direct therapy (A-II).

Rationale

As mentioned in Chapter 3, the two most basic requirements for a diagnostic test in general are analytical validation and clinical validation (7). Analytical validation demands that the test be shown to accurately identify the analyte of interest under defined test conditions. For clinical validation, the test result must accurately reflect the clinical presentation. FDA has published a guidance document specific to development of genetic tests for drug-metabolizing enzymes (8) that contains useful recommendations for the types of data needed to adequately demonstrate analytic and clinical validity. A guidance document, "*Pharmacogenetic Tests and Genetic Tests for Heritable Markers*"(9) contains additional recommendations for development of pharmacogenetic tests.

For some pharmacogenetic tests that may be specific to particular therapies (eg, Herceptest for Herceptin therapy), safety and effectiveness should be demonstrated through clinical trials of the test and drug in combination. For other types of pharmacogenetic tests that affect common drug disposition pathways, such as those that detect alleles of metabolic enzymes that will result in higher or lower than expected drug levels, a pre-existing knowledge of the effect of the enzyme variations encoded by the different alleles will frequently serve as a good starting ground for demonstrating safety and effectiveness. Further validation through drug/device trials may be required, depending on the expected change in therapy, and its effect on therapy safety and efficacy.

d) How should test and drug studies be linked in order to gain drug/test co-labeling?

Recommendation 5
Include the pharmacogenetic test in its finished form with regard to critical components in the drug clinical trial to demonstrate that the use of the test affects therapy in the intended manner (B-II).

Rationale

If a test is intended to appear in the label of one or more drugs, the test optimally will have been used in its finished form in the drug clinical trial to stratify patients or dose in the same way that it will be recommended to be used in the drug label. For example, a test to exclude patients with high risk of an adverse drug reaction should have been used in the drug trial either to exclude these patients or to identify such patients and demonstrate that safety would have been improved if the identified patients had been excluded. Tests that are either not in finished form (ie, elements of the test such as alleles tested or technology used are subject to change after the trial is complete) or that are not used in the same manner as intended for the marketed drug/test combination will likely require additional studies to ensure that the differences will not affect the ability of the test to perform as intended.

When a pharmacogenetic test has been used in a form other than final, or when the test has been developed without specific reference to a drug trial, extreme care should be taken to demonstrate that the finalized test performs as intended using the same (or closely matched) samples as were generated in the drug trial. Retrospective testing has certain limitations, such as sample selection bias, that are often difficult to overcome. When it is known or suspected that a clinical trial test will be altered prior to being offered in its final form, test developers should plan ahead and make every attempt to archive clinical trial samples in a way that will allow validation of the final test performance. For example, clinical trial testing on tumor samples may consume the smaller tumor samples, and development of the final test could only be assessed against a biased subset of samples. When a test is developed without reference to a drug, but will subsequently be used to direct therapy, test performance should be measured against samples that closely resemble the drug trial patient specimens (eg, same population distribution, same disease state and stage, same environmental factors).

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GLOSSARY

Definitions Useful in Understanding Pharmacogenetics

Daniel H. Farkas

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allele: any of several forms of a gene that is responsible

amplicon: copy of a target DNA created by an in vitro replication method

central dogma (of molecular biology)**:** fundamental tenet of molecular biology stating that DNA is copied via replication, RNA is derived from DNA via transcription, and protein is derived from RNA via translation. The discovery of reverse transcription disrupted the central dogma of molecular biology by showing that genetic information could also flow from RNA to DNA, not just DNA to RNA.

clinical utility: demonstrated evidence that an examination has potential benefit(s) in diagnosis, therapy, propensity towards developing symptoms or a disease or as an aid in a patient's or family's life choices, such as for family planning

complementary DNA (cDNA): DNA produced using an RNA template via the enzyme reverse transcriptase

compound heterozygote: individual with two abnormal alleles at a given locus, each with a difference polymorphism or mutation.

Deletion: mutation resulting from the removal of base(s)

DNase: enzyme that degrades DNA

DNA polymerase: enzyme that uses DNA as a template to produce a complementary strand of DNA; cDNA is made with a type of DNA polymerase called reverse transcriptase (an RNA-dependent, DNA polymerase)

DNA sequencing: base-by-base determination of the exact sequence of target DNA

Epigenetic: referring to heritable changes to the genome that do not alter the DNA sequence

extensive metabolizers: individuals with the normal complement of 2 fully active enzyme gene alleles (not a universally accepted definition, NACB recommends alternative nomenclature be developed)

frame-shift mutation: insertion or deletion of base(s) that alters the reading frame of a coding sequence, thereby changing the amino acids encoded downstream and/or producing a stop codon

gel electrophoresis: Separation of molecules in an electric field within a given matrix according to size and charge.

gene: segment of DNA transcribed into RNA that is translated into a protein or forms structures such as ribosomes

gene duplication: an error in DNA replication that results in two genes; often associated with the extensive metabolizer phenotype

genetic variant: structural difference in a gene which may or may not contribute to an altered phenotype

genome: all the genetic material of an organism

genotype: The genetic makeup of an organism, or group of organisms, with reference to a single trait, set of traits, or an entire complex of traits; the specific allelic composition of a gene, or set of genes, established at the DNA level

gold standard: designated reference standard or designated reference procedure

haplotype: analogous to genotype, haplotype is the set of alleles (or SNPs) on one chromosome or part of a chromosome that are linked and usually or often inherited together

heterozygote: individual with one normal and one variant allele at a given chromosomal locus

homozygote: individual with two normal (homozygous wild type) or two variant (homozygous variant) alleles at a given chromosomal locus

hybridization: Base pairing of complementary strands of nucleic acid by hydrogen bond formation, the binding of probe to specific nucleic acid sequences or amplification products

hybridization probe: a characterized DNA or RNA sequence which is used in a hybridization assay to identify closely related sequences within a mixture of nucleic acids

intermediate metabolizers: individuals who are homozygous for two reduced activity enzyme gene alleles or are heterozygous for an inactive allele (not a universally accepted definition, NACB recommends alternative nomenclature be developed)

missense mutation: base change resulting in coding of a different amino acid

molecular diagnostics: diagnosis of disease using nucleic acids as analytes, often used synonymously with molecular pathology

mRNA: messenger RNA, translated into protein

mutation: A variation in DNA sequence which is found in association with, or which reflects a predisposition to disease. Mutations must be distinguished from polymorphisms—DNA variants found in a population that neither harbor, nor is predisposed to disease. For pharmacogenetic polymorphisms, the NACB recommends using the terminology "genetic variant"

nucleoside: nucleotide lacking a phosphate group

nucleotide: building block of nucleic acids composed of phosphate group(s), a five- sided sugar molecule, and a nitrogenous base

null allele: (jargon) allele whose effect is either an absence of normal gene product or an absence of normal phenotypic function; inactive form of a gene

oligonucleotide: short sequence of nucleotides, often used as primers for PCR or DNA sequencing

penetrance: the frequency with which a specific phenotype is expressed by those individuals with a specific genotype

pharmacogenetics: the hereditary basis for inter-individual differences in drug response

pharmacogenomics: the convergence of pharmacogenetics and genomics used to mean the influence of DNA sequence variation on the effect of a drug on an individual

pharmacodynamics: reactions between drugs and living structures, including the processes of bodily responses to pharmacological, biochemical, physiological, and therapeutic effects

pharmacokinetics: study of the metabolism and action of drugs as they relate to the time required for absorption, duration of action, distribution in the body and method of excretion of the drug

phase I drug metabolism: biotransformations of chemicals that occur during drug metabolism. These reactions result in more polar metabolites of the original chemicals. Phase I reactions may occur by oxidation, reduction or hydrolysis. If the metabolites of phase I reactions are sufficiently polar, they may be readily excreted at this point but many phase I products are not eliminated rapidly and undergo a subsequent reaction in which an endogenous substrate combines with the newly incorporated functional group to form a highly polar conjugate.

phase II drug metabolism: biotransformations of chemicals that occur during drug metabolism; usually known as conjugation reactions (eg, with glucuronic acid, sulfonates, commonly known as sulfation, glutathione or amino acids, are usually detoxification in nature, and involve the interactions of the polar functional groups of phase I metabolites)

phenotype: the observable characteristics of an organism.

point mutation: mutation that substitutes, inserts, or deletes a single nucleotide

polymerase chain reaction (PCR): a common method of DNA amplification, utilizing pairs of oligonucleotide primers as start sites for repetitive rounds of DNA polymerase-catalyzed replication and alternating with denaturation in successive heating-cooling cycles

polymorphism: DNA sequence change found in 1% or more of individuals

poor metabolizer: individuals who lack active enzyme gene alleles (not a universally accepted definition, NACB recommends alternative nomenclature be developed)

primer: oligonucleotide used in PCR or DNA sequencing to target an area of interest

probe:relatively small piece of DNA or RNA used to find or detect a specific piece of target nucleic acid

prodrug: an inactive form of a drug which exerts its effects only after metabolic processes in vivo convert it to a usable or active form

proteomics: study of the entire complement of proteins in organisms

quantification standard: synthetic nucleic acid standard spiked into samples before processing to serve as a reference in quantitative PCR

real-time PCR: PCR in which detection of product is simultaneous with amplification

replication: process of duplicating DNA with DNA polymerase

restriction endonuclease (RE): enzyme purified from bacteria that recognizes and cleaves unique sequences

restriction fragment length polymorphism (RFLP): polymorphism that changes the electrophoretic banding pattern of DNA fragments generated by digestion with a restriction endonuclease

single nucleotide polymorphism (SNP): polymorphism that is a single base change

Southern blot hybridization: DNA detection method where digested sample is separated by electrophoresis, transferred to a membrane, and probed

therapeutic drug monitoring (TDM): branch of clinical chemistry concerned with the measurement of medication levels in blood; its main focus is on drugs with a narrow therapeutic index (ie, drugs that can easily be under- or overdosed)

therapeutic window: the dose range in which a drug is active

transcription: process of producing mRNA from a DNA template

translation: process of converting the information contained in mRNA into protein

ultra-rapid metabolizers: individuals with more than two copies of active enzyme gene alleles (not a universally accepted definition, NACB recommends alternative nomenclature be developed)

variant allele: specific alternative forms of a gene, generally causing a known alternative phenotype

wild-type: perceived typical form of an organism, strain, gene, or characteristic as it occurs in nature, as distinguished from mutant or variant forms that may result from selective breeding; because this term was derived from plant and other breeding studies the application of this term in human medical genetics is problematic and there is debate as to what constitutes "wild-type" when there are multiple variants that occurred naturally throughout human evolution (nonetheless, the term persists). NACB recommends that alternative nomenclature be developed for use in laboratory medicine.

APPENDIX

We thank contributors and secondary reviewers for their time and expert analysis.

Table A1. Contributors and Secondary Reviewers

Contributors	Secondary Reviewers
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